ON THE DEVELOPMENT OF THE CEREBELLUM OF THE TROUT, SALMO GAIRDNERI

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"Marvellous are Thy works,

and that my soul knoweth right well"

Psalm 139: 14

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I INTRODUCTION

The cerebellum has been the subject of a large number of anatomical and histological studies. Most of these studies deal with the mature structure in mammals. For investigation of the development of the cerebellum mammals have been the favourite vertebrate group as well.

The structure of the mature teleostean cerebellum has been described by a number of authors among whom Schaper (1893, 1894b), Franz ('11), Ariëns Kappers *et al.* ('36) and Pearson ('36) may be mentioned. Larsell ('67) and Nieuwenhuys ('67) have recently reviewed the anatomical knowledge of the teleostean cerebellum.

The ultrastructure of the teleostean cerebellum has received little attention so far. Kaiserman-Abramof and Palay ('69) presented a survey of the electronmicroscopy of the cerebellum of the mormyrid Gnathonemus, and Nieuwenhuys *et al.* ('74) studied some aspects of the synaptology in the same species. Waks ('71) described the ultrastructural characteristics of the neurons, found in the cerebellum of the trout.

Investigations on the development of the teleostean cerebellum are scarce. Schaper (1894a, b) thoroughly studied the morphogenesis and histogenesis of the cerebellum in the trout. From later years the papers of Franz ('11), Palmgren ('21), Rüdeberg ('61), Danner ('72) and Danner and Pfister ('73), dealing with several aspects of cerebellar development in teleosts, may be mentioned. The results of these investigations will be discussed in the following chapters, in direct connection with my own observations.

The aim of the present study is to give a survey of the development of a teleostean cerebellum at all levels, from morphogenesis to cytogenesis. The trout was selected for this study since several investigators have studied the same species. Moreover, the trout is generally considered as a relatively simple teleostean fish. Where possible, morphogenesis and histogenesis are

considered in their mutual relationship. The results of the histogenetic analysis comprise lightmicroscopical and electronmicroscopical findings. Both neurogenesis and gliogenesis were studied.

It will be shown that the cerebellum of the trout, although its form remains rather simple, develops into a highly differentiated structure, most of its neuronal and glial elements being directly comparable to those in mammals. The results will be discussed in light of current neuroembryological views and concepts.

II MATERIAL AND TECHNIQUES

The material consisted of embryos or brains of Salmo gairdneri RICHARDSON, 1836. The length of the animals ranged from 4.5 to 230 mm. Trout of about 140 mm are considered adult. The difference in length between successive stages was 1-2.5 mm for animals up to 25 mm, about 5 mm for animals of 25-60 mm, and 10 or more mm for larger animals.

Fish were bred as described previously (Pouwels and Smulders-Kersten, '73). Previous to fixation, fish were anaesthetized in a 0.025% solution of M.S. 222 (Sandoz), or a 0.5% solution of urethan. Embryos were directly placed in the fixation fluid. As soon as the eggs were hardened, their tough outer membranes were removed. Up to a length of 70 mm the trout were fixed by immersion. The fixation of larger animals was generally performed by perfusion through the heart. For the Golgi techniques immersion fixation was employed.

Material for *lightmicroscopy* was processed as follows. Fish, smaller than 50 mm were fixed in Bouin's fluid, dehydrated and embedded in paraffin. The material was sectioned at 7 µm for staining with haematoxilin-eosin (H.E.), and at 12, 15 or 20 µm for staining according to Bodian or Nissl. Of each stage at least one sagittal and one transversal series, stained according to each of these three techniques, were prepared. Fish, larger than 50 mm were fixed in Bouin, Susa or Bodian's fixative, and stained according to Nissl, Klüver-Barrera or Bodian. In addition, some Häggquist series of trout of 140 mm were prepared. Sectioning of the brains of trout larger than 50 mm occurred in the sagittal, transversal or horizontal plane. From fish smaller than 20 mm, two specimens of each stage were prepared by the rapid Golgi technique. At least four series were made of trout of about 20, 25, 30, etc. mm each. Eighteen series were available of trout of 140 mm. The rapid Golgi technique was performed with single or double impregnation. In addition some

Golgi-Kopsch series (of 90 and 140 mm trout) and one Golgi-Cox series (140 mm trout) were prepared. The Golgi material was sectioned at a thickness of 80 µm in the sagittal or transversal plane.

Material to be used for *electronmicroscopy* was prepared as follows. Embryos or brains were fixed with a 3% glutaraldehyde solution in 0.1 M phosphate buffer (pH 7.2). The cerebellum with surrounding tissue or, in large animals, parts of the cerebellum were dissected out. These pieces were rinsed in a 2:1 mixture of a 0.1 M phosphate buffer and a 25% sucrose solution, and then postfixed with a 2% solution of OsO_4 in 0.1 M phosphate buffer. After dehydration the pieces were embedded in Epon 812. At least 4 animals of each stage were prepared in this way. The blocks were sectioned in sagittal, transversal and horizontal planes. Sections of 1-3 µm thickness, unstained or lightly stained with a 1% solution of toluidin blue, were examined with the phase microscope. Ultrathin sections were stained with uranyl acetate and lead citrate and examined in a Philips EM 200 electron microscope.

In order to elucidate the spatial configuration of the cerebellum and adjacent structures during development, three-dimensional reconstructions including the mesencephalon and rhombencephalon were prepared of the following stages: 7, 10.5, 11.5, 14, 15, 16 and 23 mm. All of these reconstructions are based on transverse H.E. stained series. The outlines of every third section were drawn at a magnification of 190 x. The drawings were transferred to styrofoam plates of 4 mm thickness, and then these plates were cut with a hot wire along the outlines of the drawings. Subsequently the plates were stacked, using sagittally sectioned series of the same stages as a reference. In addition a three-dimensional reconstruction was made of the brain of a trout of 230 mm at a magnification of 40 x.

The terms sagittal, transversal and horizontal are used with respect to the main longitudinal axis of the brain.

ABBREVIATIONS

ang lat	angulus lateralis of the ventricle in the isthmus region
a octlat	area octavolateralis
ap	apical
astr ep	astrocytoid ependymal cells
ax	axon(s)
ax pl	axonal plexus
bas	basal
bl v	blood vessel
can cb	canalis cerebelli
caud	caudal
cb	cerebellum
centr	centriole
c fr sp	cell free space
cil	cilium
cl f	climbing fibre
comm cb	commissura cerebelli
corp cb	corpus cerebelli
cr cb	crista cerebellaris
cran cv	cranial cavity
d	dendrite
d c	"dark cell"
dec n tr	decussatio nervi trochlearium
em gr	eminentia granularis
ER	endoplasmic reticulum
eu	eurydendroid cell
ext pr	external process
f b	fibre bundles
f f	fine filaments
fil	filopodia
fiss postlat	fissura posterolateralis
fiss rh mes	fissura rhombo-mesencephalica
fov isth	fovea isthmi
G	Golgi complex
g c	growth cone
gf	gliofilaments
gg l	ganglionic layer
Go	Golgi cell
g p	glial process
gr	granule cell
gr d	dendrite of granule cell
gr l	granular layer
intaur gr bd	interauricular granular band
int pr	internal process
lat c gr	lateral cell group
lat th	lateral thickening of the corpus cerebelli
lob vestlat	lobus vestibulolateralis
lob vestlat p med	lobus vestibulolateralis pars medialis
m	mitochondrion
med c gr	medial cell group
mes	mesencephalon
m f	mossy fibre
mit	mitotic cell
m L	matrixzone L
m l g	membrana limitans gliae
m M	matrixzone M

mn	mantle cell
mn l	mantle layer
mol l	molecular layer
m P	matrixzone P
mt	microtubules
mx l	matrix layer
N	nucleus
nb	neuroblast(s)
nu cb	nucleus cerebelli
nucl	nucleolus
P	Purkinje cell
P d	dendrite of Purkinje cell
p f	parallel fibre
rec lat	recessus lateralis ventriculi quarti
rib	ribosomes
rostr	rostral
SER	smooth endoplasmic reticulum
s mx	secondary matrix cell
s mx l	secondary matrix layer
st	stellate cell
tect mes	tectum mesencephali
tegm mes	tegmentum mesencephali
tel	telencephalon
t chor	tela chorioidea
valv cb	valvula cerebelli
ventr	ventricle
v m p	velum medullare posterius
z a	zonula adhaerens

III THE CEREBELLUM OF THE ADULT TROUT; general orientation and terminology

The cerebellum of the trout comprises three main parts (fig. 1):

a. the strongly developed *corpus cerebelli*, arching over the fourth ventricle,

b. the *valvula cerebelli*, a relatively thin-walled structure that extends into the ventricle of the midbrain,

c. a transversely oriented structure, the *lobus vestibulolateralis*. The caudal border of this lobe is formed by the velum medullare posterius and the tissue surrounding the lateral recesses of the fourth ventricle. Its rostrolateral parts are known as *eminentiae granulares*.

Each of these three parts consists of three layers (figs. 1b, 2):

a. the *granular layer*, containing numerous small neurons, the granule cells; most of the afferent and efferent fibre bundles of the cerebellum pass in this layer.

b. the *ganglionic layer*, containing large neurons, most of them being Purkinje cells.

c. the *molecular layer*, mainly built up by processes of the cells present in the ganglionic and granular layers.

Reference to figures 1 and 2 shows that in the corpus cerebelli the molecular layer encloses the two other layers. In the median plane of this part of the cerebellum (figs. 1b, 2b, c) a very narrow ventricular cavity is visible, the canalis cerebelli. This canal passes between the molecular and granular layer. A ganglionic layer is not present in the median plane. At both its rostral and caudal end the cerebellar canal is continuous with the fourth ventricle. The cerebellar canal may be obliterated in places.

The valvula cerebelli is a folded structure, that passes caudally into the corpus cerebelli and dorsally into the tectum mesencephali. Laterally, the caudal part of the valvula is continuous with the tegmentum mesencephali, as shown in fig. 2a. The valvular folds enclose extensions of the cranial

cavity (figs. 1b, 2a). In the valvula the largest part of the granular layer is situated lateral to the ganglionic and molecular layers.

According to Larsell ('67) the vestibulolateral lobe comprises the eminentiae granulares, the pars medialis and the auricles. The eminentiae granulares are bilateral masses of granule cells, which occupy a superficial position (figs. 1a, 2b). The pars medialis (fig. 1b) consists of a molecular layer, through the ventral part of which fibre bundles, originating from the brain stem, pass. The molecular layer turns into the cerebellar crests (fig. 2b). The pars medialis is delimited from the corpus cerebelli by the fissura posterolateralis (fig. 3g). At both caudolateral ends of the pars medialis a mass of granule cells is present. Larsell termed these masses, which border upon the lateral recesses, auricles. The auricles are interconnected by chains of granule cells, constituting the interauricular granular band (fig. 3g). These granule cells are considered to represent the granular layer of the pars medialis of the vestibulolateral lobe.

It should be noted, that in other vertebrate groups the auriculae cerebelli are rostrolateral expansions of cerebellar tissue, surrounding extensions of the fourth ventricle.

Apart from the parts mentioned by Larsell ('67), I consider parts of the ganglionic and granular layers as belonging to the vestibulolateral lobe (fig. 2b). Processes of the cells of these layers constitute the molecular layer of this lobe. The perikarya of these cells are largely situated rostral to the molecular layer. The relations of the layers of the vestibulolateral lobe with the layers of the corpus cerebelli and with the brain stem are as follows. The molecular layer passes dorsally into the molecular layer of the corpus (fig. 1b), and ventrolaterally into the cristae cerebellares (fig. 2b). The ganglionic layer, which lies subjacent to the molecular layer (fig. 2b), passes dorsally into the ganglionic layer of the corpus and ventrolaterally into the area octavolateralis. The granular layer, which lies subjacent to the

ganglionic layer, is dorsally and rostrally continuous with the granular layer of the corpus (figs. 2b, 1b), and laterally with the eminentiae granulares (fig. 2b).

The so-called auricles and the interauricular granular band consist of granule cells that are produced rather late in development, as will be described in chapter V.

The origin and destination of fibre bundles in the cerebellum are left out of consideration in the present study.

Introduction

The morphological development of the cerebellum of the trout has been described by Schaper (1894a, b) whose findings are recapitulated in most books and articles dealing with cerebellar development (Ariëns Kappers *et al.*, '36; Larsell, '67; Nieuwenhuys, '67). Schaper's description can be summarized as follows.

In an early embryonic stage an infolding of the brain wall appears just in front of the rhomboid fossa. In this stage the very thin roof of the fourth ventricle passes into the roof of the mesencephalon. The infolding starts on both sides in the lateral parts of the brain wall and continues dorsally in the transversal plane. The caudal wall of this "Kleinhirn-Mittelhirnfalte" represents the bilaterally symmetrical anlage of the cerebellum. When the lateral parts of this fold have sharply delimited the rhomboid fossa from the more rostral parts of the brain, its dorsal part is still very shallow in the dorsal region (roof plate), but during further development the dorsal part of this furrow deepens progressively. The "Kleinhirn-Mittelhirnfalte" is initially oriented from dorsorostral to ventrocaudal; however, its position gradually changes and finally it is oriented from dorsocaudal to ventrorostral. Due to this change in orientation the cerebellar primordium is going to cap the rostral part of the rhomboid fossa. In the midline region the infolding starts to extend rostroventrally underneath the tectum of the mesencephalon, giving rise to the primordial valvula cerebelli. The next event is the extensive thickening of the cerebellar anlage on both sides of the median plane. In this way the so-called "Lateralwülste" are formed.

Later, the thickening "Lateralwülste" fuse in the median plane, separating a canalis cerebelli from the rest of the fourth ventricle. The cerebellar canal communicates with the ventricular cavity at the transition of corpus and

valvula. The caudal margin of the cerebellum passes over into the roof of the fourth ventricle *via* the velum medullare posterius. In the young embryo this structure starts to grow ventralward. Later, the corpus cerebelli grows caudally behind the primary most caudal part of the anlage. The valvula passes gradually into the tectum mesencephali. The transitional area between these two structures represents the velum medullare anterius. During development the valvula becomes thrown into folds. The most lateral parts of the cerebellum are thickenings, projecting from the brain stem. Their caudal parts surround the lateral recesses.

Some years after Schaper, Franz ('11) introduced the term eminentiae granulares for the rostrolateral parts of the cerebellum and this name has been maintained since.

Later studies on cerebellar morphogenesis and morphology in teleosts deal also with comparative aspects. A diversity of opinions is expressed concerning the question which parts of the cerebellum should be considered as auricles (Palmgren, '21; Ariëns Kappers *et al.*, '36; Pearson, '36; Larsell, '67; Nieuwenhuys, '67). In this and the next chapter we will pay special attention to the development of the lateral parts of the cerebellum.

Observations

In the youngest stage studied, an embryo of 4.5 mm (11 days), the cerebellar primordium has already been formed and consists of a transversely oriented plate (fig. 4). The dorsal part of the tectum mesencephali is thin and the tectal halves reach rather far laterally. The fissura rhombo-mesencephalica has been formed, delimiting the caudal wall of the tectum from the cerebellar anlage. Dorsally, however, this fissure is still very shallow.

Sections of 5 mm embryos (12 days) revealed that the cerebellar anlage starts to assume an orientation, directed from dorsocaudal to ventrorostral. This orientation is most pronounced in the median area. At the ventricular

side of the tegmentum a transversely oriented groove is visible, especially in the para-median regions, marking the boundary plane between mesencephalon and rhombencephalon as has been indicated already by von Kupffer ('06) and Palmgren ('21). This is the sulcus intra-encephalicus posterior. At the level of this groove the most rostral parts of the cerebellar anlage pass laterally into the tegmentum. We consider this groove to be an important landmark since the continuity between cerebellum and tegmentum at this level is maintained throughout development. The rostral tip of the cerebellar anlage will develop into a rostral direction but a fusion with the midbrain does not occur. Thus, although considerable transformations take place, the original relations are not changed.

In the 6 mm embryo (14 days) the fissura rhombo-mesencephalica has become deeper, especially in the median plane. The orientation of the cerebellar primordium is more oblique than in the preceding stage; consequently, the recessus laterales of the fourth ventricle are narrowed. Just rostral to the lateral recesses bilateral thickenings appear on the brain wall, which represent the first parts of the vestibulolateral lobe. During further development these thickenings expand rostrally.

The morphology of the cerebellar anlage of a 7 mm embryo (15 days) (figs. 3a, 5) has changed little from that of the 6 mm stage. The fissura rhombo-mesencephalica is somewhat deeper. The first fold of the future valvula has been formed, the ventral lamina being continuous with the anlage of the corpus and the dorsal lamina passing into the tectum mesencephali. The deepest part of the fissura rhombo-mesencephalica no longer indicates the external boundary between mesencephalon and rhombencephalon and, strictly speaking, the name of this fissure is no longer correct. The mesencephalic wall near the boundary with the primordial valvula is thinner than the adjacent cerebellar wall and will thin out during further development, until it finally consists of a single layer of cubic cells, the velum medullare anterius (cf. figs. 3a-d).

In the 9.5 mm embryos (18 days) all features described for the 7 mm embryo are more pronounced. In addition, the middle parts of both cerebellar halves that will constitute the corpus cerebelli, are clearly thickening, thus forming the "Lateralwülste" of Schaper.

These thickenings are more pronounced in the 10.5 mm embryos (22 days) (fig. 7c). Due to the outgrowth of the lateral thickenings, the lateral recesses become more clearly delimited from the remainder of the fourth ventricle.

In the 11.5 mm embryos (25 days), two new fissures appear, which have been indicated in figure 3c as a and b. The first one develops at the site where the velum medullare posterius passes into the tela chorioidea of the fourth ventricle. The appearance of this fissure is due to the fact that the morphologically most caudal part of the cerebellum is starting to grow ventralward, as already described by Schaper (1894b). The second fissure (b) is a transversely oriented groove situated in the middle of the cerebellar anlage. This new fissure, which is most pronounced in the midline region, indicates the boundary between the corpus and the valvula cerebelli. The lateral thickenings of the corpus project downward, narrowing the fourth ventricle.

In the next stage studied, the 13 mm young trout (hatchlings, specimens of 27 days), the lateral thickenings have further increased in size. They either have just fused in the median plane or are about to do so. The fusion will not become complete as a narrow canalis cerebelli will persist (fig. 10). These events are well described by Schaper (1894b).

As regards the sulcus intra-encephalicus posterior, the observations made by Palmgren ('21) could be confirmed. In the stage under consideration the medial part of that sulcus has fused with the fovea isthmi (fig. 3d). The lateral part of the sulcus has disappeared by now; yet its place remains marked by a small dorsoventrally oriented cell-free zone. In still later stages the rostral tip of the nucleus lateralis valvulae reaches exactly as far as this

zone and thus can be considered as indicating the boundary between mesencephalon and rhombencephalon.

In young trout of 14-15 mm (2 days), the corpus cerebelli attains its typical shape, arching above the fourth ventricle from which it is separated by the now considerably deepened fissure a (fig. 3d). The fissure designated with b in fig. 3c is deeper than in the preceding stages. The valvula still consists of a single fold.

In the period ranging from 2 to 9 days post-hatching, the size of the animals remains about 15-16 mm. During that time morphogenesis is arrested, while histogenetically, considerable progress is made. It is in this period that the various cell layers differentiate (cf. chapter V).

During later development the corpus grows caudalward, arching over the fourth ventricle. The valvula is thrown into folds, those in the area adjacent to the tectum mesencephali appearing latest (fig. 3e-g). Outgrowth of the rostral tip of the valvula rostral to the level of the sulcus intra-encephalicus posterior starts when the young trout is about 17 mm long. In 16 mm trout the velum medullare posterius has made a curve caudally (fig. 3e). Consequently, a small ventral structure becomes delimited from the dorsal corpus cerebelli by a fissure. This fissure is termed the fissura posterolateralis. The ventral structure is the pars medialis of the lobus vestibulolateralis. The latter lobe increases in size during further development, as do the other parts of the cerebellum (fig. 3f, g).

Discussion

Comparison of my results with those of earlier investigators, in particular Schaper (1894a, b), reveals that they correspond to a large extent. Nonetheless, some comments are in order.

In Schaper's view the fissura rhombo-mesencephalica ("Kleinhirn-Mittelhirnfalte") is the result of an infolding of the wall of the brain. In my

opinion, the way in which this fold develops is the same as in other vertebrates including man, *viz.*, by the lateral outgrowth of the rostral part of the rhombencephalon as well as of the mesencephalon. Since the transitional area, the isthmus rhombencephali, is not involved in these expansions, a fissure develops between the two parts. My observations are in accordance with those of Ballard ('73) who gave a general description of the development of salmonid embryos. Although the primary fissura rhombo-mesencephalica is the result of a process of lateral outgrowth of two adjacent parts of the brain wall, the deepening of the fissure is effected by the outgrowth of its bordering tissue in a ventrorostral direction.

As regards the fissura rhombo-mesencephalica my observations are at variance with those of Palmgren ('21). According to him this fissure always marks the boundary between rhombencephalon and mesencephalon, a new fissure developing between the ventral and dorsal lamina of the first valvular fold. I remained unable to distinguish two different fissures, although I admit that the name of the fissure ("rhombo-mesencephalica") no longer fits when the first fold of the primordial valvula has been formed (7 mm stage).

The boundary between corpus and valvula is indicated by a transversely oriented groove developing in the middle of the cerebellar anlage. So, the valvula is not only derived from the rostral tip of the anlage, as has been suggested by some authors (e.g. Nieuwenhuys, '67).

The sulcus intra-encephalicus posterior of the brain stem (or the structures that indicate its place after the sulcus has disappeared, *viz.* a cell-free zone and later on the rostral tip of the nucleus lateralis valvulae) indicates the level of the rostral tip of the valvula in embryos and young trout up to a length of 17 mm. In these stages the growth of the valvula more or less keeps pace with the development of the brain stem. The largest part of the valvula is formed after the 23 mm stage (fig. 3f, g) when the relative size of the corpus has reached its maximum.

The development of the vestibulolateral lobe will be discussed in the next chapter.

Introduction

The histological development of the early neural tube in general and of the cerebellum of the trout in particular has been described by Schaper (1894a, b, 1897). Schaper's opinion on the structure of the early neural tube will be discussed in chapter VII. His description of the histogenesis of the cerebellum of the trout can be summarized as follows. The early, plate-like, cerebellar anlage consists of proliferating epithelial cells. At a certain time these epithelial elements start to produce a new type of cells, which Schaper designated as "indifferent cells". These indifferent cells will form the mantle layer, situated peripheral to the germinal zone of epithelial elements. However, in the median region or "roof plate" no mantle layer is produced. Here the epithelial cells maintain their contact with both the ventricular and the meningeal surface. Proliferation of the epithelial cells and formation of a mantle layer are most pronounced in the middle parts of each cerebellar half; in this way the lateral thickenings come about. The indifferent cells are capable to divide as well as to differentiate into either neurons or glial cells. Some indifferent cells will be present even in the adult trout, but for the greater part they differentiate into the neurons and glial cells of the Purkinje and granular layers.

After formation of the mantle layer most of the epithelial cells disappear; their function as supporting cells is taken over by the glial elements. The epithelial cells that border the ventricle develop into ependymal cells. However, the epithelial cells of the velum medullare posterius, of the lateral recesses and of the "roof plate" show a long-lasting proliferative activity. Most indifferent cells produced in these regions migrate away from their sites of origin and are going to occupy a position directly underneath the external limiting membrane. Thus, these cells together constitute the "superficial

granular layer". This layer is distinguishable shortly before hatching of the trout embryos. Its cells migrate inward and differentiate for the greater part into neurons and glial cells of the Purkinje and granular layers. Consequently, the cells of the superficial granular layer are considered equivalent to the cells of the mantle zone.

Schaper further described the cerebellum of young trout of 3 to 6 months old (1893, 1894a, b). In the layer of Purkinje cells of these specimens he distinguished the following four cell types:

1. Purkinje cells. Their "protoplasmic processes" (= dendrites) extend in the sagittal plane, and form part of the molecular layer. The axons of these elements run horizontally in the layer of Purkinje cells over a wide distance, and then pass into the fibre bundles of the granular layer. In their course the axons ramify; some collateral branches enter the molecular layer.
2. Fusiform cells with two protoplasmic processes that run horizontally in opposite directions. Branches of these processes ramify in the molecular layer. This type of cell was also observed in the lower part of the molecular layer. In Schaper's opinion these elements are equivalent to the basket cells of mammals.
3. Indifferent cells.
4. Glial cells, the processes of which represent the Bergmann fibres. The radial striation shown by the molecular layer is effected by the Bergmann fibres and the protoplasmic processes of Purkinje cells.

In the granular layer of young trout Schaper also recognized four cell types:

1. Granule cells, the three to four protoplasmic processes of which are short and provided with terminal claws. These elements are confined to the granular layer. The axon of a granule cell generally arises from a protoplasmic process, and ascends to the molecular layer. In that layer the axon bifurcates T-shaped. The two branches run in the transversal plane. The horizontal striation of the

molecular layer is effected by these branches.

2. Neurons, supposed to be Golgi cells, although they are also found between and above the Purkinje cells. Their protoplasmic processes ramify in the molecular layer, their axons presumably in the granular layer.

3. Indifferent cells.

4. Glial cells, that send irregularly shaped processes in all directions.

Fibre bundles fan out in the granular layer.

Apart from the elements mentioned above, Schaper found in the molecular layer terminal ramifications of fibres of unknown origin and stellate cells. These neurons have processes in several directions, their axons could not be followed. They are supposed to originate from the superficial granular layer. The superficial granular layer was no longer observed in trout of about one year old.

Since the time of Schaper the views on the structure and the development of the cerebellum are changed on some points and amplified on others. Most of the pertinent literature deals with mammals or birds.

According to Cajal ('11) the Purkinje and Golgi cells ("large neurons") and all neuroglial elements are derivatives of the early neuroepithelium, whereas the granule and stellate cells ("smaller neurons") originate from the superficial granular layer. More recent studies, using autoradiographic techniques (Miale and Sidman, '61; Fujita *et al.*, '66; Fujita, '67, '69; Altman, '72a, b, c), have largely confirmed the opinion of Cajal. It is supposed that in addition to neurons some glial cells originate from the superficial granular layer as well.

As regards the cerebellum of the adult trout, Franz ('11) mentions the presence of "grössere Assoziationszellen" in the layer of Purkinje cells and in the granular layer. In Franz' view these, often bipolar, cells serve as links between Purkinje cells.

There is a diversity of opinion in the literature, concerning the question

whether in teleosts a cell mass, comparable to the deep cerebellar nuclei of other vertebrates, is present. Franz ('11) described a nucleus of "Ursprungszellen" at the base of the cerebellum, medial to the eminentia granularis. In his opinion the axons of these cells constitute an efferent system, the tractus cerebello-tegmentalis. Pearson ('36) made similar observations. According to the description of that author the nucleus cerebelli is situated "slightly frontal to the eminentia granularis and caudal to the cerebellar commissure. It is placed lateral to the ventricle and in close apposition to the cerebellar gray". The axons of its constituent cells would form, together with the axons of Purkinje cells, the tractus cerebello-tegmentalis and cerebello-motorius. Rüdeberg ('61) on the other hand, was not able to identify a nucleus cerebelli in teleosts with certainty; nor was Larsell ('67), except for adult Salmo gairdneri.

As mentioned in the introduction of the preceding chapter, several opinions have been expressed concerning the question, which parts of the teleostean cerebellum are comparable to the auricles of other vertebrate groups. Herrick ('24) held that the eminentiae granulares must be regarded as such. However, in the views of Pearson ('36) and Larsell ('67) only small masses of granule cells, bordering upon the lateral recesses, represent the auricles. The auricles as described by Palmgren ('21) comprise lobi externi and lobi interni. The lobi externi are the cerebellar crests; the lobi interni the lateral parts of the lobus vestibulolateralis (Larsell, '67, cf. chapter III), except for the eminentiae granulares.

From this survey of literature it appears, that there exists no unanimity concerning the structure and development of the teleostean cerebellum. Hence, the main problems investigated in the present histogenetic study are as follows:

- In which way are the cerebellar layers formed?
- Which cell types are present, where do they originate and how do they develop?
- Is a nucleus cerebelli present and, if so, how does it develop?

- In which way are the lateral parts of the cerebellum formed?

Where necessary, new terms will be introduced. The migration paths of cells in the developing cerebellum will be described in terms of the natural coordinate system. This system is formed by the ventricular and the meningeal surfaces, and by structures that extend between these surfaces (cf. Nieuwenhuys, '74). Migration may occur parallel to these structures (radial migration), or parallel to either the meningeal or the ventricular surface (tangential migration).

Observations

The histogenesis of the trout cerebellum may be divided in a period of early development and a period of later development. The early development is characterized by the formation of the three cerebellar layers, the later development by growth.

A Early development

I will start my description with some general information and subsequently a number of developmental stages will be discussed in some detail.

In the early histogenesis two phases were distinguished.

1. *The first phase.*

The earliest cerebellar anlage is a pseudostratified epithelium of proliferating cells; following Fujita ('62) we will call these elements matrix cells. The matrix consists of 5 to 6 rows of nuclei, the cells extending over the whole width of the wall. A part of the wall, *viz.* the paramedian region on both sides of the median plane, will retain these characteristics permanently. From here on this region will be called *matrixzone M.* In all other parts of the cerebellar wall a mantle layer, situated peripheral to the matrix layer, will be formed. After formation of the mantle layer in these regions the matrix no longer occupies the whole width of the wall and is termed then

ventricular matrix. During further development the largest part of the ventricular matrix is used for the formation of a mantle layer and is gradually exhausted. However, in some places the ventricular matrix is not entirely exhausted, but persists as a layer of proliferating cells. This holds for the matrix surrounding the lateral recesses (*matrixzone L*), and the velum medullare posterius (*matrixzone P*) which connects the matrices of the lateral recesses. The ventricular matrix delivers the cells of the mantle layer in a radial direction. These cells will be termed *mantle cells*, since a distinction between neuroblasts and glioblasts could not be made.

In summary, the first phase of early histogenesis is characterized by a) the formation of a mantle layer peripheral to the matrix layer, and b) the exhaustion of the matrix in certain regions.

2. *The second phase*.

This phase commences before the first phase has been completed and is characterized by the formation of a secondary matrix. Newly produced matrix cells of the matrixzones M, L, and P migrate away from their sites of origin towards the regions where a mantle layer previously had been formed. These migrating cells are termed *secondary matrix cells*. Migration occurs in a tangential direction, in a radial direction, or in both directions. The migration paths of the cells, formed in the first and second phases of histogenesis, are described in detail below, while dealing with the subsequent developmental stages.

Embryo 5-6 mm (12-14 days)

In this stage the formation of a mantle zone has just started, its thickness increasing from 1 layer of cells in the 5 mm stage to 3-4 layers in the 6 mm embryos. The thickness of the matrix layer remains 5-6 rows of nuclei. A cell-free marginal zone is not present in this stage, nor in later stages; mantle cells are observed in close proximity of the meningeal surface.

As in other vertebrates, matrix cells have elongated nuclei which are rather darkly stained by haematoxylin. Mitotic cells occupy about 50% of the ventricular surface. No exact counts were made. Considering the mitotic activity during development, it appears that 50% is about the maximum proliferative rate at the ventricular surface. This percentage decreases gradually. In the 5-6 mm embryos a few mitoses were observed in the outer part of the matrix layer. The plane of division of most of these cells is parallel to the ventricular surface; at the ventricle, on the other hand, the plane of division of mitotic cells is predominantly perpendicular to the surface.

Mantle cells are more loosely arranged than matrix cells; the nuclei of the former are rounder than those of the latter. A few mitoses were observed in the mantle layer. This is not a phenomenon restricted to the cerebellar anlage. In embryos of about 6 mm long mitoses were frequently observed in the mantle layer of the brain stem.

Semithin epon sections yielded the following additional information. The cytoplasm of the soma and of the proximal processes of many mantle cells is darkly stained (fig. 6). "Dark cells" lie also scattered among the matrix cells; in that case their nuclei may be somewhat rounder than those of the other cells in the matrix layer. "Dark cells" are found in other parts of the brain, e.g. the brain stem, as well. The dark colour in epon sections is due to the previous fixation with OsO_4. However, the structures responsible for staining could not be detected lightmicroscopically.

It has been mentioned in the chapter on morphogenesis, that in the 6 mm embryos a thickening appears on the brain wall just in front of the lateral recesses. The mode of development of this thickening is schematically represented in fig. 14c, showing a somewhat older developmental stage. The orientation of the matrix cells at the lateral recesses is such that they produce mantle cells in a predominantly rostral direction. On the contrary, the matrix cells at the angulus lateralis of the ventricle in the isthmus region emit the

mantle cells laterally. These migrations lead to the formation of an eminence on the external brain wall. This eminence is not to be confused with the eminentia granularis, that is not formed until the second phase of histogenesis. However, both structures will form part of the lobus vestibulolateralis, as will be pointed out later on.

Embryo 7 mm (15 days)

In this stage the matrix layer is thinner but the mantle layer is thicker than in the preceding one. Both consist of 4 to 5 rows of nuclei. A distinct boundary between these two layers cannot be drawn. The matrix layer shows considerable mitotic activity. We estimate that slightly more than 50% of the ventricular surface is occupied by dividing cells. The number of "extraventricular" mitoses is larger than in 6 mm embryos. These mitoses are found in the matrix layer as well as in the mantle layer and in the latter, even close to the meningeal surface.

The axons of the cells of the nucleus nervi trochlearis have entered the cerebellar anlage. The decussation of these axons appears as a very small bundle subjacent to the rostral tip of the rhombomesencephalic fissure, as is shown in fig. 3b.

Embryo 9.5 mm (18 days)

The first blood vessels have entered the cerebellum from the meningeal surface. The matrix layer is built up by about four rows of nuclei and the mantle layer by about six rows. At the ventricular surface the mitotic activity is somewhat less than in the preceding stages, but is still considerable. Several "extraventricular" mitoses are present throughout the matrix. In this stage only very few mitoses were observed in the mantle layer, which holds true for the mantle layer of the brain stem as well. "Extraventricular" mitoses are easily distinguished from dividing blood cells, which are also found.

Blood cells are identified by their clear cytoplasm and distinct cell membrane.

Embryo 10.5 mm (22 days)

This stage is much more advanced than the preceding one. In the region where the lateral thickenings develop the ventricular matrix consists only of 1 to 2 rows of nuclei; here the mantle layer is about 14 rows of nuclei thick (fig. 7c). In the vicinity of the matrixzones M, L and P the ventricular matrix is thicker whereas the mantle layer is thinner (fig. 8) than in the area of the lateral thickenings. Many of the mitoses are situated at the ventricular surface; "extraventricular" mitoses are found throughout the matrix and sometimes in the mantle layer. The plane of division of mitotic cells is variable. Even at the ventricular surface this plane may be not only perpendicular to the surface, but also parallel to it or somewhat oblique. All these positions of the plane of division are found among the "extraventricular" mitoses as well.

The first phase of histogenesis is not yet finished in this stage. However, the appearance of the earliest secondary matrix cells indicate the outset of the second phase. These cells are recognized as dividing cells in the vicinity of the matrixzones M, L and P. Figure 8 shows some secondary matrix cells near the meningeal surface. The morphology and migration paths of secondary matrix cells are described in the next stages. Leaving the few secondary matrix cells aside, the 10.5 mm embryos have been used to give a schematical representation of the first phase of histogenesis (fig. 14). Migration of mantle cells occurs radially only. The most conspicuous parts of the mantle layer are:

- The lateral thickenings in the region of the future corpus cerebelli (figs. 7c, 14a).
- The eminence on the external brain wall in front of the lateral recesses, described already in the 6 mm stage (fig. 14c). In 10.5 mm embryos fibre bundles

have divided the rostral part of the eminence into a medial and a lateral cell group. This is also shown in figure 7a, a transverse section at the level of the fibre bundles. Figure 7b shows the eminence immediately behind the fibre bundles.

New features of the 10.5 mm stage are furthermore:

- The appearance of the commissura cerebelli (figs. 3b, 7b), a decussation situated in the caudal part of the primordial valvula.
- The occurrence of cell-free spaces in the lateral thickenings (fig. 7c).

These spaces occur at those places where the matrix has become very thin. Blood vessels are always present at the borders of these spaces. Danner ('73) and Pfister and Danner ('74), who deny the presence of such spaces before hatching (i.e. 13 mm stage), called them "subependymale Cysten". However, since a cyst should possess a wall of its own which is absent here, we prefer the term cell-free spaces. Similar spaces are observed in the brain stem, as early as the 9.5 mm stage. A close relation seems to exist between the incidence of these spaces on the one hand, and the presence of blood vessels and the absence of a matrix layer of some rows of nuclei on the other hand.

Embryo 11.5 mm (25 days)

In this stage the lateral thickenings are strongly developed (fig. 9). In the region of these thickenings the matrix consists of a single layer of cells. As shown in figure 9, these matrix cells are no longer tightly packed; sometimes they are only connected at their base (the ventricular side) by fine processes. Mitoses are still observed in this layer. Only very few mitoses are found in the mantle layer. Semithin epon sections revealed no "dark cells" in the lateral thickenings.

More secondary matrix cells are produced than in the preceding stage. They have the same staining qualities as the matrix cells in the zones M, L and P. Their nuclei tend to be slightly rounder than the nuclei present in

those matrixzones. Since the 15 mm stage was chosen to illustrate the second phase of histogenesis, the migration paths of secondary matrix cells are described there.

Blood vessels are numerous; the cell-free spaces have increased in size. Some material, which seems to be derived from degenerated cells, is present in these spaces. The velum medullare posterius contains a bundle of transversely oriented fibres. These could not be traced beyond the cerebellum.

Young trout 13 mm (hatchling)

The first phase of histogenesis has come to an end. Except for matrixzones L and P the ventricular matrix is exhausted. In some 13 mm trout the lateral thickenings have expanded to such an extent that they are fusing in the median plane (fig. 10). In other trout this fusion takes place in a later stage. The site of fusion includes the areas where the cell-free spaces are present.

Some mantle cells, particularly those near the matrixzones M, L and P, show signs of differentiation. These elements have a slightly larger and lighter staining nucleus than the other cells in the mantle layer (fig. 11). It is plausible that they represent neuroblasts.

Secondary matrix cells may form a separate layer near their sites of origin. In the case of secondary matrix cells originating from matrixzone M (fig. 11) this layer is about three rows of cells thick. These cells migrate tangentially under the meningeal surface. The plane of division of mitotic cells in this layer is predominantly oriented either perpendicular or parallel to the surface. Cells resembling secondary matrix cells are found in the mantle layer; it is concluded that these elements are migrating radially after a previous tangential migration. Some of these cells still appear to be capable of mitosis. Semithin epon sections of the 13 mm stage revealed the presence of "dark cells" (fig. 12). It is remarkable that most secondary matrix cells

possess a dark cytoplasm. Some cells in the matrixzone and some cells in the mantle layer are also darkly stained. In my opinion the dark cells observed in the mantle layer are derivatives of the secondary matrix.

The first trace of a molecular layer appears in 13 mm trout. This layer develops between the mantle layer and the secondary matrix.

The decussation of the axons of the cells of the nucleus nervi trochlearis no longer occupies a position subjacent to the rostral tip of the rhombomesencephalic fissure. This fibre bundle is slightly displaced in a caudoventral direction. During later development the decussation is further displaced and finally lies about halfway the valvula (fig. 3b-g). The fibre bundles in the velum medullare posterius have increased in size.

Young trout of about 15 mm (2-9 days)

Some days after hatching growth of the young trout shows an arrest, which extends over about one week. During this period the formation of the cerebellar layers, i.e. molecular, ganglionic and granular layer, is established. Leaving neuroglia aside, I determined that in general:

- the ganglionic layer develops from the mantle layer
- the granular layer originates from the secondary matrix
- the molecular layer is built up by processes of neurons in the ganglionic and granular layers.

Exceptions to these general rules will be mentioned in the following analysis. The way in which the cerebellar layers are established is elucidated in figure 15, representing a survey of the second phase of histogenesis. In this survey the differences in histogenesis among the various regions of the cerebellum are shown. These regions will now pass in review.

The corpus cerebelli (fig. 15a). Secondary matrix cells originating from matrixzone M first migrate tangentially over some distance. Subsequently, these elements migrate radially and finally they settle near the ventricular surface.

These cells differentiate into granule cells. In regions where the lateral thickenings have been fused, granule cells will form a mass of cells in the core of the corpus cerebelli (fig. 13b). The cell-free spaces are gradually filled up.

Most mantle cells will occupy a position peripheral to the granule cells, but a number of them are found within the developing granular layer. The neuronal mantle cells differentiate synchronously with these granule cells into the large neurons of the cerebellum. Those situated in the granular layer develop predominantly into Golgi cells, all others into Purkinje and eurydendroid cells, i.e. the neurons of the ganglionic layer. The development of these cell types will be described in detail later on. The earliest differentiating large neurons are recognized in those areas where the secondary matrix is most strongly developed, i.e. in the vicinity of the matrixzones. Here the first parts of the molecular layer appear as well, since the development of this layer keeps pace with the development of the ganglionic and granular layers.

The valvula cerebelli (fig. 15b). A number of secondary matrix cells follow migration paths comparable to those described above. However, since the ventricle between valvula and brain stem is rather narrow, the capacity of this region for accomodating cells is limited (fig. 13a). Many secondary matrix cells perform tangential migration and settle then as granule cells lateral to the developing ganglionic layer (figs. 13a, 16). As in the corpus, some mantle cells occupy a position within the developing granular layer and differentiate into Golgi cells.

The region rostral to the lateral recesses (fig. 15c). Secondary matrix cells produced by matrixzone L migrate radially, i.e. in the same direction as the mantle cells that previously have been formed by this matrixzone. The derivatives of the secondary matrix cells, i.e. the granule cells, settle rostral to most of these mantle cells. The so-called eminentia granularis develops from the lateral portion of these, rostrally migrating granule cells.

Caudal to the granular layer the ganglionic and molecular layers differentiate, in the same fashion as has been observed in other parts of the cerebellum. Around the angulus lateralis of the ventricle the ganglionic and molecular layers are continuous with the rhombencephalic area octavolateralis and crista cerebellaris, respectively; all of these structures develop during the same period. The mantle cells situated rostral to the developing granular layer, were produced in the first phase of histogenesis, by those ventricular matrix cells, that surround the angulus lateralis of the ventricle rostral to the lateral recesses. Fibre bundles have split up these mantle cells in a medial and a lateral group. The lateral cell group and the ventral part of the medial cell group will develop into rhombencephalic nuclei. The dorsal part of the medial cell group (fig. 16) will differentiate into the nucleus cerebelli described by Pearson ('36). Matrixzone L passes medially into matrixzone P. The structures produced by both matrixzones are therefore also continuous. The derivatives of these matrixzones together constitute the lobus vestibulolateralis, the development of which will be described later on.

The region bordering upon matrixzone P (fig. 15 d1-4). Matrixzone P passes medially into matrixzone M (d1) and laterally into matrixzone L (d4). The migration paths of cells originating from these two zones are described above. From medial to lateral the orientation of the matrix cells in zone P turns about 180 degrees. As depicted, the migration paths of the secondary matrix cells change direction in the following manner:

- Medially, the number of cells performing tangential migration first, increases (fig. d2).
- Laterally, the number of cells performing radial migration only, increases (fig. d3). After migration through the mantle layer bordering upon matrixzone P, the secondary matrix cells take part in the building of the cerebellar granular layer. The mass of produced granule cells passes continuously over into the eminentia granularis laterally and in the granular layer of the core

of the corpus cerebelli medially. As elsewhere, the mantle cells between granular layer and secondary matrix differentiate into Purkinje and eurydendroid cells. Simultaneously, a molecular layer develops between the secondary matrix and the ganglionic layer.

In 16 mm (9 days) trout histogenesis has proceeded to such a degree, that the three cerebellar layers can be clearly recognized. No cells have fully differentiated as yet. Cresyl violet series reveal the presence of only little Nissl substance in the neurons of the ganglionic layer. The Golgi technique, applied to the stages discussed so far, yielded negative results in the cerebellum, no cells being impregnated.

Compared with younger stages number and size of fibre bundles in the cerebellum have much increased. It is beyond the scope of this investigation to trace the origin or destination of these fibres. The reader is referred to the work of Pearson ('36) and the reviews of Ariëns Kappers *et al.* ('36), Nieuwenhuys ('67) and Larsell ('67).

The part of the valvula near the velum medullare anterius is much retarded in development. In the 16 mm stage it consists only of a matrix, starting to produce mantle cells. The development of this part of the cerebellum is not followed in the present study; in the adult it has the same construction as the rest of the valvula.

B Later development

This section traces the subsequent histological and cytological changes of the cerebellum in its transition to the mature structure. The events taking place will be described under the following headings: a) the development in general; b) the development of the lobus vestibulolateralis; c) the development of the nucleus cerebelli, and d) the development of the neuronal and glial cell types.

a) *The development in general*

When the cerebellar layers essentially have been formed, further development is characterized by growth (cf. fig. 3e-g). General trends are the increase in thickness of the granular and molecular layers and the thinning out of the ganglionic layer. Apparently no new large neurons are added to the last-mentioned layer and the available cells have to cover an increasing area. However, the individual neurons show considerable increase in size. The granular layer thickens by growth of its constituent cells, particularly their processes, but also by addition of new cells, which originate from the secondary matrix. The thickening of the molecular layer is chiefly due to the increase in number and size of its constituent structures: processes of the cells of the ganglionic and granular layers; also, new cells, originating from the secondary matrix, are added to it.

The tangentially migrating cells of the secondary matrix gradually extend further away from their sites of origin, but an uninterrupted layer under the meningeal surface is never formed. In the adult trout a superficial discontinuous layer is present; its constituent cells should perhaps still be considered as matrix cells. Semithin epon sections of 22 mm (23 days) trout revealed that "dark cells" are the secondary matrix cells and their derivatives in the molecular and ganglionic layers. The cytoplasm of the soma and the proximal processes is darkly stained. These observations correspond to those in the hatchlings.

b) *The development of the lobus vestibulolateralis*

The lobus vestibulolateralis is the derivative of matrixzones L and P (fig. 15c, d). The three cerebellar layers: molecular, ganglionic and granular are clearly present in this lobe. These layers pass medially into the corresponding parts of the corpus cerebelli.

A clear boundary between lobus vestibulolateralis and corpus cerebelli

can only be drawn for the molecular layer. This boundary is the fissura posterolateralis (fig. 3f, g), which is most pronounced in the median region; the fissure comes about by a curvature of matrixzone P after the 15 mm stage. In median and paramedian sections the molecular layer of the vestibulolateral lobe appears very thick (fig. 3f, g). Due to the complex relations in the median region the molecular layer is cut here more or less tangentially (cf. fig. 2b).

As pointed out previously, a close relation exists between the lobus vestibulolateralis on the one hand, and the areae octavolaterales with their cerebellar crests on the other hand. During the first phase of histogenesis the matrix cells surrounding the angulus lateralis of the ventricle near the lateral recesses produce a continuous mantle zone (fig. 7a, b). The dorsal part of this zone will develop into the ganglionic layer of the cerebellum, the ventral part into the cell masses of the area octavolateralis (fig. 17). Axons of granule cells of the eminentia granularis participate in building the cerebellar crest. The cerebellar crest passes continuously into the molecular layer of the lobus vestibulolateralis (fig. 2b).

The production of granule cells by matrixzones P and L extends over a long period. In young trout of about 30 mm total length granule cells are found bordering upon the matrixzones, i.e. in the same area as the secondary matrix cells. These granule cells have differentiated *in situ*, without preceding migration through the molecular layer. Their number increases with age. Their axons constitute the most caudal part of the molecular layer of the vestibulolateral lobe. Pearson ('36) termed these granule cells of the lateral recesses together the auricles. He pointed out that these cells are interconnected by the stratum granulosum pars ventralis. Larsell ('67) termed these structures auricles and interauricular granular band respectively.

c) *The development of the nucleus cerebelli*

The origin of the cells of the nucleus cerebelli has already been described. During the period of early development these cells remain densely packed (figs. 7a, b, 16). In later stages the cells cover a larger area, their arrangement being looser. In accordance with the description given by Pearson ('36), the cells finally occupy a position near the tractus cerebello-tegmentalis and cerebello-motorius. It is difficult to demarcate the nucleus cerebelli from the adjacent cells in the brain stem, some of which perhaps constitute the secondary gustatory nucleus as described by Barnard ('36). In two sagittal Golgi series of 24 mm trout some cells were partly impregnated in the region where the nucleus cerebelli is located. The axons of a few of these elements could be followed into the brain stem.

In adult trout the size of the soma of the cells of the nucleus cerebelli is smaller than of the cells of the ganglionic layer.

d) *The development of the neuronal and glial cell types*

The development of the cerebellar neurons and neuroglial elements could partly be studied in the Golgi material. Most cytological details will be given in the chapter on electronmicroscopy. The Golgi technique yielded positive results in the cerebella of trout larger than 20 mm.

The development of the following elements will be described successively:

1. neurons
   a. of the ganglionic layer
   b. of the granular layer
   c. of the molecular layer
2. afferent fibres in the cerebellum
   a. mossy fibres
   b. climbing fibres
3. neuroglial elements

Where possible, the description of developmental events is preceded by a brief characterization of the adult structure in question.

1 a. *Neurons of the ganglionic layer*

The neurons of the ganglionic layer are the Purkinje and the eurydendroid cells, the former being the most numerous. A mature *Purkinje cell* is depicted in figure 18. The dendritic tree is largely oriented in a plane perpendicular to the orientation of the parallel fibres (see section 1b, granule cells). Soma and primary and secondary dendritic branches are rather smooth, but the branches of higher order are densely covered by knoblike protrusions, the dendritic spines. The axon is largely confined to the ganglionic layer, giving off a number of collaterals (fig. 19). Some of these collaterals pass towards the molecular layer to branch there.

Cresyl violet series reveal the presence of clumps of Nissl substance in the soma, most of which are either concentrated around the nucleus or at one side of the cell. In a sagittal Klüver-Barrera series 50 Purkinje cells (in which the nucleoli were visible) were randomly selected for measuring the sizes of both soma and nucleus of these cells. The somata measured from 22 by 16 to 13 by 12 μm (mean value about 17 by 15 μm). The average diameter of their rounded nuclei appeared to be 9.5 μm. Two nucleoli per nucleus were frequently observed.

The development of Purkinje cells is represented in figure 20. The immature Purkinje cell of figure 20a shows an irregularly shaped soma, bearing several appendages. The smooth axon, only a part of which was present in the section, seems to be further advanced in development than the dendrites. A collateral of the axon is just visible. Two main dendritic trunks emanate from the soma, one of them ramifying in the molecular layer. The dendrites bear processes, which strongly resemble the filopodia described by Morest ('69a, b) for developing dendrites in the mammalian brain. In accordance with the obsery-

ations of that investigator, the terminal part of the dendrites may be enlarged, forming a growth cone with filopodia (arrow). The Purkinje cell of figure 20b is more advanced in development than the one depicted in figure 20a. Its soma bears fewer appendages; only one dendritic trunk is present, as is usual for adult Purkinje cells. The filopodia of the dendritic branches may be very long (arrow 1). In some places it seems that new dendritic branches are formed out of a growth cone and its filopodia (arrow 2). Tertiary and higher order branches bear small protrusions, resembling spines. The axon of the cell starts to branch at some distance away from the soma; in some places the axon shows a beaded appearance. The Purkinje cells shown in figures 20c and d, which have been found in somewhat older specimens, have a rather smooth soma and dendritic trunk. The length of the dendritic trunk has increased. Secondary and tertiary dendritic branches still bear many filopodia and growth cones (arrows). The axon has not changed much, compared with the cell depicted in figure 20b. Cresyl violet series of comparable stages show some distinct Nissl substance in the soma.

It is noteworthy that in the developmental stages under consideration the dendritic trees of the Purkinje cells do not extend as yet in one plane.

The development of Purkinje cells can be summarized as follows:

- The formation of the axon with its collaterals begins before the elaboration of the dendritic tree.
- Soma and dendritic trunks form slender appendages, the filopodia. Dendritic branches grow and new branches are formed at those places where growth cones are present. Possibly new branches can develop from filopodia.
- Filopodia of soma, primary and secondary dendrites disappear gradually during the development of the dendritic tree.
- The appendages of tertiary and higher order dendrites are filopodia and dendritic spines. Whether spines represent transformed filopodia or develop after the disappearance of the latter structures could not be determined. In young

adult trout filopodia are very scanty, and the dendritic shafts are densely covered with spines.

- The developing dendritic tree becomes gradually oriented into one plane.

The second neuronal type which is present in the ganglionic layer is the *eurydendroid cell* (fig. 18). The shape of the soma of these elements varies from rounded to fusiform. In general, two main dendrites arise from opposite poles of the soma and one other dendritic trunk emanates from the upper aspect of the soma. The two main dendritic trunks extend in the ganglionic layer over some distance and then enter the molecular layer to ramify there. The other dendritic trunk usually passes directly to the molecular layer. Some of the eurydendroid cells are situated in the upper part of the granular layer. The somata of these elements issue primary, secondary and even higher order ramifications before entering into the molecular layer. The dendrites of eurydendroid cells bear small spines which are placed rather far apart and are not provided with terminal knobs. The name eurydendroid cell is based upon the large extension of the dendritic tree. The orientation of most of the dendritic trees of these elements is in a plane perpendicular to the orientation of the parallel fibres, just as the dendritic trees of the Purkinje cells. A few eurydendroid cells are oriented parallel to the parallel fibres. In Golgi and Bodian preparations it was observed that somata and dendritic trunks are entwined by an axonal plexus (figs. 18, 21). The axon of eurydendroid cells originates from the soma and enters the granular layer. Unfortunately the axon could only be followed over a short distance. Since the Golgi technique often fails to impregnate myelinated fibres, it is concluded that the axon is myelinated soon after leaving the soma.

Cresyl violet series yielded the following additional information. The eurydendroid cells can be distinguished from the Purkinje cells because of the diffuse spread of the Nissl substance in their somata. The sizes of 50 eurydendroid cells

droid cells were determined in the same series as used for the Purkinje cells. Their cell bodies measure from 30 by 15 to 14 by 10 μm (mean value about 24 by 11 μm), their rounded or slightly ellipsoid nuclei about 10 by 8 μm. These results imply that:

- the size of eurydendroid cells is more variable than that of Purkinje cells,
- eurydendroid cells are on the average larger than Purkinje cells,
- the average soma/nucleus ratio of eurydendroid cells is larger than that of the Purkinje cells.

Eurydendroid cells are not unique for the trout cerebellum, the same cell type was described in the mormyrid fish Gnathonemus petersii (Nieuwenhuys and Nicholson, '69; Nieuwenhuys *et al.*, '74). In my opinion this cell type corresponds to the fusiform cells described by Schaper (1893, 1894a) (although we did not observe them in the molecular layer) and the "grössere Assoziationszellen" of Franz ('11). Probably the eurydendroid cells are not equivalent to the "cellules fusiformes horizontales", described by Cajal ('11) in the upper part of the granular layer of mammals because the somata of those cells, which are always fusiform, are smaller than the eurydendroid cells. Contrary to the eurydendroid cells, the dendritic tree of the fusiform horizontal cells is mainly confined to the ganglionic layer. Another cell type found in the upper granular layer of mammals, the Lugaro cell (see Cajal, '11; Palay and Chan-Palay, '74), differs from eurydendroid cells in having an axon which ascends into the lower part of the molecular layer and ramifies there.

Little information could be obtained on the development of the eurydendroid cell. The few impregnated elements of this type (fig. 22) are further developed than the Purkinje cells, at least with respect to the differentiation of their dendritic trees. Distinct filopodia and growth cones are not observed. The soma is rounded and of about the same size as that of a Purkinje cell. The dendritic trunks, which may be very short, do not issue from opposite poles of the cell, but yet their ramifications do already span a large part of the mole-

cular layer. In two cases (24 mm trout), the axon could be followed towards the base and even outside the cerebellum, passing with other fibres through the core of the corpus.

1 b. *Neurons of the granular layer*

The neurons of the granular layer are the granule cells and the Golgi cells.

Figure 3g reveals that the granular layer is very thick, the number of *granule cells* being much larger than that of other neurons. Mature granule cells are depicted in figure 18. The size of the soma is small in comparison to the size of the neurons of the ganglionic layer. The dendrites are smooth and have terminal claws with about three digits. The axon usually arises from a dendrite, and ascends to the molecular layer. In figure 18, which is based on sagittal sections, it is not visible that the axon bifurcates in the molecular layer, forming a parallel fibre. Parallel fibres are tangentially oriented.

The development of granule cells shows differences among the various regions of the cerebellum. However, in all regions the direction of migration and the orientation of newly formed processes follow the natural coordinate system, i.e. a tangential or a radial direction. The development of granule cells in these regions will now be described. The following terminology will be used for the processes of developing or mature granule cells:
- the process "in front of" the soma, pointing towards the path of migration, is termed the leading process (l p),
- the process "behind" the soma, in the path of migration, is termed the trailing process (t p). These two terms are adopted from the work of Rakic ('71, '72). Of the parallel fibres I distinguished two parts: Part x, situated between the bifurcation and the site of origin of the cell, and part y, being the opposite part of the parallel fibre. The axonal segment, connecting the

soma with the point of bifurcation, is designated the ascending axon (a ax).

Granule cells of the corpus cerebelli (fig. 23a, cf. fig. 15a). In 13 mm trout, when radial migration has just started, the secondary matrix layer contains many tangentially running fibres near matrixzone M. Hence, it is concluded that the future granule cell, while migrating, leaves a trailing process behind. This process becomes part x of the parallel fibre. Golgi sections of later stages revealed that part x may gradually bend radially and that part y may lie at a lower level than part x (cf. cell 2 in fig. 23a). It is probable, therefore, that in those cases part y has not grown out before radial migration has started. In young trout part x of the parallel fibre and the ascending axon together indicate the path of migration. In later stages the orientation of cells according to the natural coordinate system is for the greater part lost in the granular layer. Due to the increasing inflow of newly produced granule cells a certain proportion of these elements becomes displaced laterally. In the adult the ascending axons of laterally situated granule cells show an orientation which is no longer radial (cf. cell 2 in fig. 23a).

Parts x of many parallel fibres grow out and pierce matrixzone M from the 16 mm stage onward.

Granule cells of the valvula cerebelli (fig. 23b, cf. fig. 15b). Part of the granule cells performs tangential as well as radial migration, as in the corpus cerebelli (cell 1 in fig. 23b). Other cells, represented by cell 2, perform tangential migration only. In those cases, the parallel fibre corresponds to the trailing process, at least in young stages when matrixzone M has not yet been pierced.

Granule cells of the lobus vestibulolateralis (fig. 23c, cf. fig. 15c). Granule cells which perform radial migration only, orient themselves parallel to the velum medullare posterius after leaving the matrixzones L and P. Then, these cells start to migrate radially. During further development the three parts of the axon are lengthened simultaneously. The lateral part of many

parallel fibres, belonging to granule cells of the eminentia granularis, curve ventralward to participate in the formation of the crista cerebellaris.

In the vicinity of matrixzone M future granule cells of the lobus vestibulolateralis migrate tangentially over a short distance after leaving matrixzone P (fig. 15 d2). After having done so, they orient themselves parallel to the velum medullare posterius and continue their migration in a radial direction. The three parts of the axon of these elements grow out simultaneously.

The granule cells of the so-called auricles (see chapter III) do not migrate. These elements extend their axons parallel to the parallel fibres of the other granule cells of the vestibulolateral lobe.

The development of the granule cells in the cerebellum of the trout appears to be at variance with the development of these cells in mammals and birds, as described by Cajal ('11) (fig. 23d). According to that author, the cells of the "superficial granular layer", which originate from the region surrounding the lateral recesses of the fourth ventricle, pass through three phases before reaching their destination: 1. Phase germinale ou indifférente (1 in fig. 23d). 2. Phase de la bipolarité horizontale (2 in fig. 23d). 3. Phase de la bipolarité verticale (3 in fig. 23d). Thus, the two parts of the parallel fibre would develop simultaneously and a large part of the "ascending" axon would develop prior to the radial migration of the soma. The most superficially situated granule cells would not be provided with processes. The observations of Cajal have been confirmed by a number of authors, among whom Altman ('72a, '75), who studied the cerebellum of the rat, may be mentioned. According to del Cerro and Snider ('72) the germinal cells of the superficial granular layer in the rat have long, thin processes.

When a granule cell in the cerebellum of the trout has reached its destination in the granular layer, the soma starts to form dendrites. In 20 mm trout these processes are short and simple, and lack terminal specializations (fig. 24a). In 24 mm trout a number of granule cells appeared to have dendrites with

terminal knobs (fig. 24b), which later will develop into the characteristic claws (fig. 18).

Even in young adult trout (14 cm total length) granule cells with an immature appearance could be found. A developmental stage, in which the soma bears numerous unramified appendages, as described by Cajal ('11), was not observed in my material.

All neurons in the granular layer with dendritic ramifications in the molecular layer and axonal ramifications in the granular layer were classified as *Golgi cells*. Figure 18 (Go_1) shows a typical example. Judging from Golgi preparations, the Golgi cells represent a rather heterogeneous group, showing variations in the size of the soma, the shape of the dendritic tree, as well as the pattern of the axonal ramifications. However, the following general observations were made. 1. Golgi cells have a radial orientation, but their dendritic and axonal ramifications are not restriced to a single plane. 2. The dendrites bear small spines. 3. Some dendritic branches may be confined to the granular layer. 4. More than one axon (up to three) is usually present, as is found for Golgi cells in mammals (Palay and Chan-Palay, '74).

A number of cells in the granular layer did not meet the description of Golgi cells given above, but these neurons most probably were partly impregnated elements (fig. 18 Go_2). A very small number of cells in the granular layer could not be classified as Golgi cells, since their dendritic and axonal ramifications were confined to the granular layer. These cells were not investigated further.

The Golgi cells are derivatives of the ventricular matrix layer. A young Golgi cell is depicted in figure 25. The branching of the dendrites and axons is much less than in mature Golgi cells (cf. fig. 18 Go_1). Distinct growth cones and filopodia were not observed.

1 c. *Neurons of the molecular layer*

The stellate cells (fig. 18) are the only neurons the soma of which is situated in the molecular layer. Compared with the neurons of the ganglionic layer their size is small. Dendritic and axonal ramifications both are confined to the molecular layer. Two or more dendritic trunks issue from the soma. The dendritic branches are smooth or bear some small spines. The axon gives off many collaterals; in a number of cases it was observed that axonal branches made contact with Purkinje dendrites. The dendritic and axonal ramifications of these elements are not confined to one plane.

Although the overall shape of the stellate cells shows considerable variations, no subdivisions within the group were made so far. Basket cells, i.e. cells with axon collaterals surrounding several Purkinje somata, were not found.

A very immature stellate cell is depicted in figure 26a. The number of ramifications of its processes is much less than in the adult. Stellate cells of rather young trout are shown in figure 26b, c. It is remarkable that they are provided with very long processes, which run parallel to the parallel fibres of the granule cells. In some instances these tangential fibres branch T-shaped from a stem process (fig. 26b, c, arrows). The diameter of the long processes is always slightly larger than that of parallel fibres. Further, these processes issue side branches, particularly in their proximal parts. On the basis of these observations we exclude the possibility that the cells with long processes are displaced granule cells. Moreover, in my opinion, the tangentially-running processes are dendritic, since (1) they bear small spines, and (2) their diameter corresponds to that of short stellate cell dendrites, but is larger than that of stellate cell axons. Stellate cells with such long dendrites were not found in the adult trout so far. This means either that they were not impregnated in our Golgi series, or that the distal parts of the tangentially-running dendrites disappear as the animal reaches maturity.

2 a. *Afferent fibres in the cerebellum: mossy fibres*

Mossy fibres (fig. 18) are myelinated axons that enter the cerebellum in bundles. Their extensive ramifications are found throughout the granular layer. The characteristic formations to which these fibres owe their name, consist of short varicose arborizations and thickenings, as described by Cajal ('11) for mossy fibres in birds and mammals. Figure 27 shows that, apart from the irregularly formed central portion, very thin, often beaded appendages are present. Simpler forms with a less complex central part or without the thin appendages have also been observed. "Mossy" formations, i.e. rosettes, are found along the course of the fibre, at branching points and at the terminals of the fibre. The same three locations were distinguished by Cajal ('11).

Concerning the development of the mossy fibre rosette, the following observations have been made. In 20 mm trout simple enlargements of the mossy fibre are found (fig. 28a). These enlargements develop progressively into structures with an irregular surface: the central portions of the rosette. Central parts that have reached a certain degree of irregularity (i.e. of maturation) start to form the thin filiform appendages (fig. 28b).

2 b. *Afferent fibres in the cerebellum: climbing fibres*

Climbing fibres are afferent fibres that terminate in the molecular layer. Their terminations exclusively contact the main dendritic branches of Purkinje cells, by twisting along them in the fashion of lianes along a tree (Cajal, '11). So far no climbing fibres were found in the Golgi series of the adult trout. However, three Golgi series of trout of about 30 mm revealed the presence of climbing fibres (fig. 29). No cells were impregnated in the areas where these fibres were found, hence it could not be determined which structures are contacted by them. Their terminations are chiefly situated in the ganglionic layer, a few of them occurring in the lower part of the molecular layer. These observations suggest that the climbing fibres are mainly in con-

tact with the somata of the Purkinje cells.

Cajal ('11) distinguished three phases in the development of climbing fibres: 1. the "phase du nid" or "phase du plexus infracellulaire", in which the terminal arborizations are perisomatic, 2. the "phase de la cupule" or "phase du capuchon supra-cellulaire", in which the terminal arborizations contact the upper part of the soma and the proximal dendritic trunk, and 3. the "phase de l'arborisation grimpante jeune", in which the terminal arborizations approach their final position on the main dendritic branches of the Purkinje cells. Comparison of figure 29 with the figures given by Cajal (cf. also O'Leary *et al.*, '71) shows that the climbing fibres observed by me in young trout represent the "phase du nid" and "phase de la cupule". Thus, these fibres might still be immature.

3 *Neuroglial elements*

The cerebellum of the trout accomodates a wide variety of neuroglial cell types. Most of them correspond to neuroglial elements found in the cerebellum of mammals and other vertebrates. A few types have not been described previously, however. In the adult trout the following neuroglial elements have been observed:

a) *Golgi epithelial cells* (fig. 30). The soma of these elements is situated in the ganglionic layer. Its shape is variable and irregular, its size smaller than that of the Purkinje cells. The soma issues some processes, which ascend into the molecular layer. These processes are known as Bergmann fibres. They are studded with fine and lamellar, sometimes branching, appendages, which give the fibres a mossy appearance. The fibres reach the outer surface of the cerebellum, where they participate in the membrana limitans gliae.

b) *Ependymal cells* (fig. 31). Ependymal cells keep in contact with both the ventricular and the meningeal surface, and are found in the regions where a matrix layer persists. In figure 31 some cells of matrixzone M are depicted;

figure 31a represents a presumptive matrix cell, figure 31b the peripheral process of an ependymal cell. The main processes of ependymal cells have a more or less radial or tangential course in the molecular layer. These processes are provided with numerous side branches, the latter frequently being oriented at right angles to the former. A number of branches reaches the meningeal surface, their terminal knobs forming part of the membrana limitans gliae. Occasionally, some branches are interconnected by a very thin layer of cytoplasm; I will term these structures "veils".

c) *Ependymoid astrocytes* (fig. 32). The irregularly shaped somata of the elements of this type are situated in the ganglionic layer. Most processes ascend to the molecular layer, where they have a radial orientation. These processes display the same characteristics as the processes of ependymal cells. Other processes may be present that do not ascend to the molecular layer, but extend in the ganglionic and upper part of the granular layers. Such processes resemble the processes of velate protoplasmic astrocytes, described under d). Occasional observations were made of processes of ependymoid astrocytes contacting blood vessels (fig. 32, arrows).

d) *Velate protoplasmic astrocytes* (fig. 33). These neuroglial elements were first described in detail by Chan-Palay and Palay ('72) for the cerebellar cortex of rat and monkey. Golgi and electronmicroscopical preparations showed that the same cell type is also present in the cerebellum of the trout. Velate astrocytes are predominantly found in the granular layer, where they are numerous. The main processes are rather coarse, the higher order branches are much finer. Branching occurs predominantly "at right angles". At many places the fine processes are interconnected by a very thin film of protoplasm. In this way septa are formed, consisting of "ribs" and "veils" (arrow). Some septa together enclose a compartment. This peculiar arrangement of the processes of velate astrocytes results in a compartmentalization of the granular layer. Occasionally it was observed that velate astrocytic processes contact

blood vessels.

e) *Astrocytoid ependymal cells* (fig. 34). Neuroglial cells that line the ventricle and have their processes in the granular layer stand intermediate between ependymal cells and velate astrocytes. The proximal parts of such cells resemble ependymal cells (cf. fig. 39a), the distal parts are similar to velate astrocytes (cf. fig. 33).

f) *Smooth protoplasmic astrocytes* (fig. 35). These cells have been found in the molecular and granular layers, but they are far less common than the velate astrocytes. Their smooth processes are slender and tortuous. The branching pattern of the processes differs from that of the neuroglial elements described above since, generally, the branches are placed at acute or obtuse angles.

g) *Oligodendrocytes* (fig. 36). These cells were rarely impregnated in the Golgi series. Figure 36a shows an oligodendrocyte, the processes of which pass into the myelin sheaths of axons. The oligodendrocyte of figure 36b extends from the upper part of the molecular layer to the fibre bundles in the granular layer. In the molecular layer some processes are in contact with a blood vessel. The processes of the oligodendrocytes observed so far were smooth and slender. The branching pattern of these processes resembles that of smooth astrocytes.

h) *Microglial cells* (fig. 37). In only one series very small cells were found, which contacted a blood vessel. The shape of these cells tallies with that of microgliacytes in the toad (Stensaas and Stensaas, '68a), and hence they were tentatively interpreted as microglial cells.

Concerning the development of neuroglial elements the following observations have been made. In young trout of 20-24 mm some of the elements described above were found, namely:

- Ependymal cells (or parts of them, fig. 38a, b),
- Velate protoplasmic astrocytes (figs. 38c, 39, 40). Transitional forms between the elements represented in a and b of figure 39 have been found. The

cells depicted in figure 40, cell 8 and figure 39a probably represent successive stages of development. The element, numbered with 7 in figure 40, is possibly a very young velate astrocyte or astrocytoid ependymal cell.
- Golgi epithelial cells (fig. 40, cells 2, 4, 5, 6). The somata of these cells occupy a position in the ganglionic or in the granular layer. In some places (arrows) their processes do not show the characteristic mossy appearance: here fine branches are interconnected by veils.

Apart from identifiable Golgi epithelial cells, elements are found that resemble both young ependymal (or ependymoid astrocytic) and Golgi epithelial cells (fig. 40, cells 1, 3). A small part of one process of the element numbered 3 shows a mossy appearance (arrow), in cell 1 that feature is more pronounced.

Smooth protoplasmic astrocytes and oligodendrocytes were found only in later stages of development. In this context it is worth noting that myelinization commences when the animal attains a total length of about 25 mm.

On the basis of the observations mentioned above it is hypothesized that:
- Golgi epithelial cells, ependymal cells, ependymoid astrocytes, velate astrocytes and astrocytoid ependymal cells originate from the ventricular matrix. Ependymal cells are also produced by matrixzone M.
- Glioblasts that maintain contact with both ventricular and meningeal surface differentiate into ependymal cells.
- Glioblasts that maintain contact with the meningeal surface and generally loose their connection with the ventricular surface, develop into Golgi epithelial cells or ependymoid astrocytes.
- Glioblasts that maintain contact with the ventricular surface, loose their connection with the meningeal surface and extend their peripheral process in the granular layer, develop into astrocytoid ependymal cells.
- Golgi epithelial cells that loose contact with both the ventricular and the meningeal surface differentiate into velate astrocytes.

- The differentiation of a glioblast into any of these cell types mentioned so far is strongly influenced by its surrounding structures.

The origin of smooth astrocytes and of oligodendrocytes could not be determined. We only found these cell types in later stages of development and in the adult. If it is true that they are not present earlier, not even as glioblasts, they must derive from the secondary matrix after the formation of the main mass of granule cells.

The origin of microglial cells is unknown.

Discussion

For solving the problems mentioned in the introduction of this chapter, the understanding of two issues appeared to be essential. The first is the differential development of the matrix layer in the various regions of the cerebellum. The second is the orientation and migration paths of the cells in the cerebellum during development. The migration paths of the mobile elements depend on the orientation of the fixed cells. The above mentioned factors influence histogenesis and, to a certain extent, morphogenesis. The degree of development and the position of the layers, formed in the first and in the second phase of histogenesis, depend upon these factors.

So far, the present study did not reveal the factors involved in the curvature of the cerebellum during development. In the regions where this curvature occurs, the orientation of the axes of the natural coordinate system is altered, and hence also the direction of migration of cells and the positioning of the layers.

My results on the establishment of the cerebellar layers are difficult to reconcile with those of Rüdeberg ('61). The "migration layers", distinguished by that author in the cerebellum of some teleosts, do not correspond to the migration layers found in the present study. The term migration layer indicates a layer of cells, which have performed similar migrations. For example,

migration layer B of Rüdeberg constitutes the main mass of the granular layer as well as the layer of Purkinje cells. The present study showed that Purkinje cells and granule cells have a different origin and follow entirely different migration paths.

The studies of Danner ('72) and Danner and Pfister ('73) deal with the development of the cerebellar layers in the trout; these authors distinguished a ventricular matrix giving rise to the mantle layer in early development, and an "external granular layer" originating from the matrix in the median region and giving rise to the "internal granular layer" in postembryonal development. The presence of a matrix surrounding the lateral recesses is mentioned by Danner and Pfister. Migration of the cells of the "external granular layer" produced in these lateral regions, is thought to occur in the same way as the migration of the "external granular" cells produced by the matrix in the median region. However, the present study revealed that characteristic differences exist in the migration paths of cells produced by the matrixzones of various regions.

One of the problems mentioned in the introduction of this chapter, concerned the presence of auricles, i.e. rostrolateral diverticula of cerebellar tissue surrounding extensions of the fourth ventricle. Using that definition in the strict sense, auricles are not present in the cerebellum of the trout. As shown, the lateral recesses of the fourth ventricle caudally border upon the lateral parts of the cerebellum and do not extend within these parts. However, the close relation between the lateral recesses and those structures could be demonstrated, since the entire lateral portions, including the eminentiae granulares, are derivatives of the matrix layer adjoining the lateral recesses. In my opinion, there is no ground to designate the small masses of granule cells, which in later stages of development and in the adult border upon the lateral recesses, as auricles, as was done by Pearson ('36) and Larsell ('67). The supposition of Herrick ('24) and Ariëns Kappers *et al.* ('36)

that the eminentiae granulares expand during development to such a degree, that the lateral recesses become greatly reduced, could not be confirmed. The lateral parts of the cerebellum form part of the vestibulolateral lobe, and ventrally turn into the cristae cerebellares and areae octavolaterales of the brain stem. The auricles of the teleostean cerebellum as described by Palmgren ('21), comprise parts of the vestibulolateral lobe as well as the cerebellar crests (see introduction to this chapter). However, I do not agree that these structures should be considered as auricles.

The presence of the group of cells, designated as nucleus cerebelli by Pearson ('36), was confirmed. The origin of these cells appeared to be the ventricular matrix surrounding the lateral angle of the ventricle rostral to the lateral recesses. It should be noted that the afferent and efferent fibre connections of this nucleus were not traced in the present study.

A few remarks must be made concerning the development of the cell types. In accordance with the view of Cajal ('11), I consider the large neurons (Purkinje, eurydendroid and Golgi cells) and most neuroglial elements to originate from the ventricular matrix. The secondary matrix layer produces smaller neurons: the granule cells, and probably the stellate cells (see the next chapter), and perhaps some neuroglial cells.

The term secondary matrix was introduced, instead of the terms superficial or external granular layer or external germinal layer, which are commonly used in developmental studies of the cerebellum in general. The secondary matrix cells of the trout do not form a superficial layer in all regions (fig. 15). A further difference of the secondary matrix in the trout with the comparable layer in higher vertebrates, is its long lasting proliferative activity. The external matrix layer of mammals and birds is exhausted some weeks after its appearance, whereas the proliferative period of the secondary matrix in the trout lasts at least some months. Even in the adult trout, cells are found with the morphological characteristics of secondary matrix cells.

The differentiation of a secondary matrix cell into a neuroblast could not be followed morphologically. Even among cells, which have left the secondary matrix layer, mitoses have been observed. In this context the observations of Kranz and Richter ('70) on the cerebellum of young specimens of Lebistes reticulatus are interesting. In their autoradiographic study those authors found many DNA synthesizing cells in the molecular layer of corpus and valvula cerebelli. In their opinion these cells do not represent glial cells only. If we hold that neuroblasts do not divide, such cells in the molecular layer are still to be regarded as matrix cells. However, according to my observations migrating cells in the molecular layer are provided with axons (fig. 23).

My studies on the differentiation of the cells of the ganglionic layer (most of them Purkinje cells) and of the granule cells suggest that these two categories of cells interact. This supposition is based on the following observations: 1. The first young neurons of the ganglionic layer are found in those regions where the secondary matrix layer is best developed. 2. The dendritic trees of the neurons of the ganglionic layer develop in the same area as the axons of the granule cells, irrespective of the migrations performed by each of these neurons (figs. 14, 15). 3. The main plane of the Purkinje dendritic tree is always oriented perpendicular to the orientation of the parallel fibres. That the maturation of a neuron is induced or influenced by its afferent fibres, was suggested by a number of authors, among whom Cajal ('11) and Morest ('69a, b) may be mentioned. Recent studies of abnormal mammalian cerebella (mutants or X-irradiated animals) have amplified this view (Altman and Anderson, '72; Altman, '73; Rakic and Sidman, '73; Hamori, '73). The results from these studies on the interaction between the development of Purkinje cells and granule cells may be summarized as follows. 1. Parallel fibres exert a guiding influence on the pattern of growth of the Purkinje dendritic trees. 2. The growth of Purkinje cell dendrites shows considerable autonomy. Even in the absence of parallel fibres the characteristic spiny

branchlets are formed. On the basis of the observations presented above, the present study could confirm the first of these statements. With regard to experiments employing X-ray irradiation it should be borne in mind, that in such experiments the possibility cannot be excluded that a failure in the development of the Purkinje dendritic tree is caused directly by the irradiation, and not, as is suggested by the authors mentioned above, indirectly by the absence or impairment of the granule cells. The same applies to the mutants which were investigated.

As regards the differentiation of the Purkinje cell dendrites, the climbing fibres are considered to exert an influence on this process (Cajal, '11; Kornguth and Scott, '72; Sidman and Rakic, '73; Hamori, '73). According to Kornguth and Scott ('72) the climbing fibres induce the smooth branches of the dendritic tree, the spiny region being induced by a different agent, but Hamori ('73) emphasizes the influence of climbing fibres even on the development of the spiny branchlets (heterotopic induction). In contrast with the results of Hamori are the findings of Sotelo ('75), who failed to demonstrate any influence of climbing fibres on the maturation of the dendritic trees. Purkinje cell dendrites developed normally in rats, in which total degeneration of climbing fibres was induced before the establishment of functional synapses between these fibres and Purkinje cells. The role of climbing fibres in the maturation of the Purkinje cells in the trout could not be determined in the present study.

The analysis of the neuroglial elements in the cerebellum of the trout, has yielded some interesting results. According to Cajal ('11) neuroglia of fishes consists of "cellules ependymaires" and "cellules névrogliques ordinaires". The last mentioned term is not further explained. Studying the tectum mesencephali of some teleosts electronmicroscopically, Kruger and Maxwell ('67) distinguished ependymal cells, astrocytes and some oligodendrocytes. As shown in the present study, in the cerebellum of the trout all of the neuroglial

cell types described for the mammalian cerebellum are found (cf. Palay and Chan-Palay, '74), and in addition the so-called ependymoid astrocytes and astrocytoid ependymal cells, both combining characteristics of ependymal cells and of velate astrocytes. The occurrence of transitional forms between neuroglial cell types, possibly indicating a common precursor cell type, is frequently described in the literature (Ramon-Moliner, '58; Stensaas and Stensaas, '68c; Vaughn, '69).

Literature on the development of neuroglia (Cajal, '11; Fujita *et al.*, '66; Fujita, '67, '69; Glees and Meller, '68) reveals that in general most neuroglial elements are considered to derive from the original matrix layer (ventricular matrix), and some elements from the "external" matrix layer. According to Fujita *et al.* ('66) the latter elements represent oligodendrocytes and astrocytes, but in the view of Privat ('73) only some oligodendroglia, particularly that of the molecular layer, originates from the "external" matrix layer. As has been pointed out, smooth astrocytes and oligodendrocytes in the cerebellum of the trout possibly arise from the secondary matrix. All other neuroglial elements originate from the ventricular matrix layer. My classification of these various elements shows resemblance with that of Silver ('42), who studied the neuroglia in the spinal cord of the frog. That author distinguished 1. cells that extend from ventricle to pia (ependymal cells), 2. cells that have lost their contact with the ventricle but remain attached to the pia, and 3. cells that have lost their contact with both the ventricle and the pia.

Apart from the developmental events considered above, two phenomena observed during the maturation of the cerebellum of the trout, have to be discussed. The first is the presence of cell-free spaces, the second the occurrence of "dark cells".

Cell-free spaces are found in animals of about 10 to 16 mm, in regions where the ventricular matrix layer is nearly or entirely exhausted. Blood

vessels are present at the borders of these spaces. Danner ('73), Danner and Pfister ('73) and Pfister and Danner ('74) called them "subependymale Cysten" and ascribed a neurosecretory function to them. In my opinion, the cell-free spaces which are observed in the brain stem as well, rather result from a degeneration or disintegration process in those areas where only a few cells of a layer of previously tightly joined cells are left over. As described, the cell-free spaces are filled with granule cells during the second phase of histogenesis.

"Dark cells" have frequently been observed in the adult central nervous system of many species. Cammermeyer ('62), Friede ('63) and a number of other authors regard them as artifacts, due to the fixation procedure, post-mortem changes or mechanical damage. Friede ('63) further leaves room for the possibility that the dark cells represent a pathological condition. This view was amplified by Stensaas *et al.* ('72) who observed "dark cells" in the vicinity of mechanical lesions, in addition to dark cells being post-mortem artifacts. Some authors ascribe the appearance of dark cells to physiological changes (Tewari and Bourne, '63: Purkinje cells of the rat, Nemetschek-Gansler and Becker, '64: nuclei supraopticus and paraventricularis of the rat, Ford and Rhodes, '65: oliva inferior of the rat). Studying dark cells electronmicroscopically, Mugnaini ('65) concluded that these cells presumably result from poor fixation. He found that all dark cells had crenated outlines and swollen processes adapted to the concavities of the cell surface. Another electronmicroscopical study (Chan-Palay *et al.*, '74) suggests that dark (hyperchromic) cells are spontaneously degenerating.

On the basis of the following observations I believe that the dark cells in the cerebellum of the trout are neither artifacts, nor degenerating cells. 1. Their morphology does not correspond to Mugnaini's description. 2. The tectum mesencephali of young embryos, which is composed only of matrix cells does not contain dark cells although in the brain stem of the same young

embryos such elements are present. In the latter part of the brain a mantle layer is being formed; the dark cells are found in the matrix and mantle layer. 3. During the first phase of cerebellar histogenesis dark cells are present in the ventricular matrix and the mantle layer, except for those regions where the matrix is exhausted. 4. During the second phase of histogenesis the secondary matrix cells and their derivatives, which have not yet reached their destination, show the dark appearance. No cells of the original mantle layer are dark in that period.

From the observations summarized above, I tentatively conclude, that the dark cytoplasm, seen after osmium fixation, is a feature of cells which are migrating or are about to migrate. In this view the dark colour indicates a certain physiological condition of the cell. The dark reaction of the cytoplasm is confined to the soma and proximal parts of the processes.

Other aspects of "dark cells" will be discussed in the following chapters.

Introduction

The few published electronmicroscopical studies on the teleost cerebellum deal with the mature structure of that organ (Kaiserman-Abramof and Palay, '69; Waks, '71; Nieuwenhuys *et al.*, '74). The development of the cerebellum has been investigated ultrastructurally in other vertebrate groups, particularly mammals, with exception of the earliest development, of which no studies are known to me. For the investigation of the early neural wall other parts of the central nervous system have been selected, especially the spinal cord, the cerebral hemisphere and the retina in birds and mammals. Many of these studies will be discussed in direct connection with my own observations.

In this chapter the differentiation of the cell types is described in some detail. Special attention will be paid to the following aspects:

- The differentiation of matrix cells into neuroblasts and glioblasts.
- The synaptology and the development of the neuronal circuits.

Unless mentioned otherwise, the observations refer to the corpus cerebelli or its anlage.

Observations

The observations will be described according to the following scheme:

A. The stages from 5 to 14 mm. The mantle layer of 14 mm trout contains morphologically distinguishable neuroblasts and glioblasts. From this stage onward it is possible to describe the development of the individual cell types as follows.

B. The development of the cell types.

1. Cells of the matrixzones M, P and L, and secondary matrix cells.
2. Neurons

a. of the ganglionic layer

- differentiation of Purkinje cells and eurydendroid cells
- synaptology

b. of the granular layer

- differentiation of granule cells and Golgi cells
- synaptology

c. of the molecular layer

- differentiation of stellate cells
- synaptology

3. Neuroglial elements

A. The stages from 5 to 14 mm.

Embryo 5-6 mm

The formation of the mantle layer has started in some regions. The ultrastructure of the matrix cells in those regions differs slightly from that of matrix cells in the areas where a mantle layer is not yet present. The last-mentioned cells, which constitute a neuroepithelium, are described first. The description is based on matrix cells in the period of interphase (figs. 41, 42). In the elongated nuclei the chromatin is rather evenly distributed; an irregularly shaped nucleolus may be observed. In the cytoplasm the following organelles are found.

(1) Many free ribosomes, occurring singly, in rosettes or in small clumps.

(2) Mitochondria, often two or three lying close together. Some mitochondria are elongated and have constrictions. The last-mentioned feature may suggest division, which is supposed to be a means of increase of mitochondria (see Pannese, '74, for the various theories on this subject).

(3) One or two prominent Golgi complexes located in the internal process (i.e. the process reaching the ventricular surface, also termed the apical end).

(4) Some smooth and some rough endoplasmic reticulum (SER and RER). The cisternae of the ER are short and wide. They are found in both the internal and the external processes. Sac-like profiles in the region of the Golgi complex may bear ribosomes as well.

(5) Microtubules, with a diameter of about 200 Å; these organelles are most conspicuous in the internal process and oriented predominantly parallel to the longitudinal axis of the cell.

(6) A cilium extending from the internal process into the ventricle. Apart from a cilium the internal process bears several protrusions at the ventricular surface.

(7) A centriole at the base of the cilium; another centriole may be found oriented perpendicular to the first one.

(8) Some small electron-dense vesicular structures, surrounded by a membrane, being scattered throughout the cytoplasm.

At the ventricular surface the internal processes of the matrix cells are joined together by special junctions (fig. 42). These are largely of the zonula adhaerens type, although the width of the gap between two adjacent cells may vary in one and the same junctional complex (cf. Sheffield and Fishman, '70; Hinds and Ruffett, '71). Such distinct junctions were not observed between the external processes of matrix cells. In a few cases it was found that the membranes of the external processes of adjacent cells approach each other very closely, but no submembranous specializations were present. In other instances the external processes were joined by puncta adhaerentia (cf. Hinds and Ruffett, '71).

The matrix cells are closely packed together, but in the outer half of the wall rather large extracellular spaces may be found. This is presumably related to the fact that mitotic cells lack external processes (Sauer, '35; Hinds and Ruffett, '71; Seymour and Berry, '75).

In the outer half of the wall profiles of growth cones and filopodia are

found (see below).

The matrix layer that has commenced to produce a mantle layer shows some new features. The cells are generally less tightly packed, and their orientation is less strictly radial. The nuclei of these ventricular matrix cells have a slightly more irregular outline, sometimes with indentations (fig. 46, cell 2). Chromatin distribution is not as even as in the matrix cells described above; conspicuous clumps may be present.

"Dark cells" are found among the ventricular matrix cells and in the mantle layer. Some "dark cells" are in contact with the ventricle, but most of them have lost this contact. In the latter the organelles surround the nucleus. The dark aspect of "dark cells" is due to two factors. First, free ribosomes occurring predominantly singly, are numerous and are evenly distributed throughout the cytoplasm. Second, the cytoplasm has a dark appearance by the presence of extremely fine filamentous material.

The mantle layer was studied in the next stage.

Embryo 7 mm

Mantle cells differ from ventricular matrix cells in some respects. Their somata and their nuclei are more or less rounded, and their cytoplasmic organelles generally occupy a perikaryal position (fig. 46, cell 3). The same organelles are present as in matrix cells, even a pair of centrioles. However, cilia could not be demonstrated in mantle cells. Sometimes accumulations of vesicular and tubular structures were observed under the surface membrane of the soma (growth areas). In other cases such accumulations occurred in cone-like protrusions of the surface membrane. These structures are termed growth cones.

Between the mantle cells axonal profiles are encountered (these are found between the ventricular matrix cells as well). They contain the following organelles: (1) Microtubuli, oriented parallel to the longitudinal axis of the

axon. (2) Some vesicular endoplasmic reticulum. (3) Some mitochondria. (4) A few ribosomes. (5) Very few microfilaments.

A few terminals participating in the formation of the membrana limitans externa show some differentiation. Compared to the external processes of matrix cells, those terminals have a clear aspect due to a smaller amount of ribosomes. Further, the number of microtubules is decreased, but particularly conspicuous is the presence of microfilaments. Further development shows clearly that these features are characteristics of glioblasts. So we may conclude that the first visible glioblasts participate in the formation of membrana limitans externa. Unfortunately the processes of glioblasts could not be followed to their perikarya. Thus, although clear cytological differences between mantle cells could not be observed, a distinction between neuroblasts and glioblasts may be made on the basis of the structure of their processes (fig. 46).

Some submeningeal terminals extend fine processes under the surface, the filopodia. These structures are described in the next stage in which they are more distinct.

Embryo 9.5 mm

The nuclei of some peripherally situated mantle cells have a slightly more regular outline than in the preceding stage. In the mantle layer the number of areas with accumulations of vesicular and tubular structures, i.e. growth areas and growth cones, is increased. They are found in the somata and in the processes, and are numerous in the submeningeal terminals as well. Filopodia extend from these terminals and are also observed extending from axonal profiles. Filopodia are slender processes, characterized by the presence of extremely fine filamentous material and generally contain no other organelles. Filopodia and growth cones are shown in figure 43.

Embryo 10.5 mm

As described in the previous chapter, the 10.5 mm embryos are considerably more advanced in development.

Generally, the amount of smooth endoplasmic reticulum is increased in all elements of the mantle layer. Rows of vesicular SER are observed in connection with the Golgi complex, near the periphery of the cells and in their processes as well. In this and in the following stages myelin figures, consisting of a whorl of membranes, were occasionally found in a soma or a process. The perikarya of a few mantle cells include more elongated cisternae of RER than are found in matrix cells and in the other mantle cells, but further differences among the mantle cells were not observed.

In this stage axonal and glial processes could be distinguished. In glial processes microtubules are only found occasionally, these organelles being characteristic for neuronal processes (fig. 46). Microfilaments are more numerous in the glial processes, particularly in their submeningeal terminals, than in the neuronal processes. The existence of dendritic processes could not be established with certainty. A few profiles, containing some more SER and free ribosomes than is usual for axons, possibly represent dendrites.

Puncta adhaerentia are found between all types of structures in the cerebellar anlage.

In the region of the lateral thickenings the ventricular cells may have filopodia along the ventricle. Many submeningeal terminals possess very long filopodia, the proximal parts of which may contain some SER and free ribosomes. Probably these filopodia fill up places where cells have retracted themselves from both the ventricular and meningeal surface.

In summary, this stage gives the impression of extensive growth of processes, and only very slight progress in the differentiation of the somata.

Embryo 11.5 mm

In this stage young dendrites could be distinguished. They contain the same organelles as axons but, with the exception of microtubules, in larger numbers (fig. 46). The diameter of the microfilaments, present in axonal, dendritic and glial processes, is about 80 Å. Such filaments are most numerous at the basement membrane of the submeningeal terminals. All of these terminals display the characteristics of glial processes.

For the first time glycogen granula are found. Large numbers of these granula are present in glial processes. They also occur in small amounts in neuronal and glial perikarya and in dendrites as well. Since glycogen granules appeared to be absent in some other series, the presence of glycogen is at best an auxiliary criterion in the identification of the neuronal and glial structures. Figure 44 shows the different types of processes in the mantle layer.

Many cells, some of them bordering the ventricle, contain cisternae of endoplasmic reticulum which are longer than those in matrix cells. The cell-free spaces appear to be filled with granular material and a few indefinable, small membrane-bound structures. The secondary matrix is described in the next stages.

Young trout 13 mm (hatchling)

In this stage the first synapses have appeared. In accordance with the work of Molliver and van der Loos ('70), Armstrong and Johnson ('70) and del Cerro and Snider ('72), the following criteria were used to identify a synapse: (1) specializations at one or both membranes, (2) at least two or more vesicles with the characteristics of synaptic vesicles in one of the two processes involved, (3) separation of the two membranes by a clear gap. Since no thorough analysis of synaptogenesis was carried out, membrane specializations alone, which a number of authors consider as indicating the first synaptic differentiation (Aghajanian and Bloom, '67; Bunge *et al.*, '67; Meller and Haupt, '67;

Mugnaini, '69; Larramendi, '69; Stelzner *et al.*, '73), were not taken into account to preclude confusion with other junctional complexes. However, I tend to agree with these authors, that paramembranous specializations develop prior to the appearance of synaptic vesicles. It could not be determined whether synapses develop from puncta adhaerentia, or whether they are entirely new formations.

The few synapses found in this stage, are exclusively axodendritic. They occur on dendritic shafts. Dendritic spines are not yet present. The junctional specializations vary from symmetrical to asymmetrical. The synaptic vesicles are round or nearly round, with a diameter of about 400 Å . They are preferentially situated near the thickenings of the presynaptic membrane. In the same axonal profile more than one synaptic specialization may be found. In a few cases it was observed that an axonal growth cone had formed a synapse, as found in the developing cerebellum and spinal cord of the chick (Foelix and Oppenheim, '74; Oppenheim *et al.*, '75). According to the observations of those authors, the first synapses in the chick include large dense-cored vesicles. Such vesicles were not present in the earliest synapses in the cerebellum of the trout. Generally, the presynaptic component of these synapses contained no other organelles than synaptic vesicles and a few tubules of SER. In later stages a mitochondrion and slightly more organelles may be found. Comparison with later stages showed that the first synapses in the cerebellum of the trout occur between parallel fibres and Purkinje cell dendrites.

In 13 mm trout it appeared to be possible to distinguish some neuroblasts from the other cells of the mantle layer. These neuroblasts (fig. 46, cell 4) are somewhat larger than mantle cells. Their rounded nuclei are less electron-dense, the chromatin is evenly distributed and the nucleolus is distinct. In the cytoplasm more RER is present with longer membranes than in mantle cells. The number of free ribosomes has decreased.

Secondary matrix cells closely resemble the cells of the matrixzones, al-

though the distribution of the organelles may be different. Contrary to the observations of del Cerro and Snider ('72a) and Altman ('72a) in the rat, microtubules are present in the secondary matrix cells. Further, as described in the previous chapter, these cells are "dark". The dark appearance is due to an abundance of free ribosomes and to the presence of extremely fine filamentous material, as was observed in areas in the cell where the ribosomes were less densely packed.

As regards filaments, two types are recognized (fig. 45). The first consists of distinct filaments with a diameter of about 80 Å (microfilaments). They occur in glial processes and in smaller amounts in neuronal processes in 13 mm trout. In later stages these glio- and neurofilaments are also found in the somata. The second type consists of finer filaments, often even extremely fine, which generally form a network in all processes and somata. The network is loosely meshed in most structures, i.e. somata, processes, growth cones. However, it is much denser in "dark cells", in filopodia and in mitochondria. In the extracellular space some of such fine filaments may be found as well.

The extracellular space is far less wide in the 13 mm trout compared with the 11.5 mm embryos.

Young trout 14 mm

In 14 mm trout synapses are still scarce and exclusively axodendritic. Paramembranous specializations are asymmetrical.

Puncta adhaerentia are rarely found on the surface of young neurons, but they do occur on other cell types.

Many somata of the mantle layer are not yet interpretable as either neuronal or glial. However, some glioblasts could be identified by following a typical glial process to its soma. These glioblasts (fig. 46, cell 5) resemble mantle cells, but their cytoplasm contains slightly more RER with elongated cisternae and fewer free ribosomes. Just as mantle cells, the glial somata are

provided with microtubules, although these structures are almost entirely absent in their processes.

The findings on the development from matrix cell to recognizable neuroblast and glioblast have been summarized in figure 46.

B. The development of the cell types

1. *Cells of the matrixzones M, P and L, and secondary matrix cells*

The matrixzones display more or less the same characteristics in all stages. During the period of strong proliferation they contain large amounts of ribosomes while chromatin is very conspicuous in most of their nuclei.

There tends to be an increase in number of junctional specializations in the matrixzones with age. The gap between the opposite membranes of these junctions may vary in width and is in some places even obliterated. In young stages such junctions were only present near the ventricle, but later on these specializations occur elsewhere too.

Curved bar-bell shaped mitochondria are frequently present in the cells of the matrixzones. Such a morphology could suggest that these mitochondria are dividing (cf. Pannese, '74).

In specimens of 34 mm and longer, it was observed that some cells in the matrixzones had differentiated into ependymal elements.

As has been mentioned in the previous chapter, a varying number of cells in the matrixzones are very dark, while other cells have a lighter appearance. Similar shades in electron density have been observed among the secondary matrix cells near their site of origin. However, at some distance from their site of origin, *all* of the secondary matrix cells are very dark. The ultrastructure of the secondary matrix cells is essentially the same as that of the cells of the matrixzones, although the distribution of the organelles may be different.

Contrary to the condition in mammals (Cajal, '11; Mareš *et al.*, '70; del

Cerro and Snider, '72; Altman, '72, '75), the secondary matrix layer of the trout never forms the outermost zone of the cerebellum. The latter is composed of glial terminals. Three morphologically separate layers, as found for mammals and birds, could not be distinguished in the secondary matrix of the trout (fig. 23). It was found that tangentially and radially oriented secondary matrix cells both are found immediately under the membrana limitans gliae (fig. 47).

Many of the secondary matrix cells appear to have a distinct process, which contains the same organelles as the soma, in the direction of the site of origin of the cell. Such processes, not observed in continuity with their soma, are also found between the cells. These structures probably correspond to the trailing process described in the previous chapter. In addition, the secondary matrix contains tangentially-running axonal profiles (fig. 45). These are observed already in 11.5 mm embryos directly under the membrana limitans gliae. The leading processes of secondary matrix cells are shorter than the trailing processes. In some cases it was observed that such a leading process terminated as a growth cone.

Small junctional specializations are present between the secondary matrix cells, but submembranous densities are inconspicuous. In the external matrix layer of the rat Zagon and Lasher ('72) found junctions of the zonula adhaerens type. As in mammals, synapses do not occur in the secondary matrix layer of the trout. In older specimens, when this layer consists of scattered cells, these elements do not only form junctions with each other, but with their surrounding structures, particularly glia, as well.

An interesting observation is made in trout of 15 mm and more. In these stages bundles of parallel fibres have pierced matrixzone M. A considerable part of the newly-produced secondary matrix cells migrates laterally under these bundles of parallel fibres. Since many of these elements will differentiate into granule cells, the opinion held for mammals, that later coming

granule cells have their parallel fibres at a higher level in the molecular layer, does not hold for the trout. Figure 48 shows a tangentially oriented secondary matrix cell in the middle of the molecular layer, which has almost completed mitosis.

2 a. *Neurons of the ganglionic layer*

The differentiation of mantle cells into neurons of the ganglionic layer is characterized by the following features:

1. The nucleus becomes larger and less electron-dense, with an even distribution of chromatin.
2. There is a decrease in the number of free ribosomes.
3. There is an increase in the amount of RER and in the number of mitochondria. The RER cisternae appear narrower and more elongated. Sometimes membranes of the RER are observed in continuity with the outer nuclear membrane (fig. 50).

In the first days post-hatching, groups of neurons lie close together. Puncta adhaerentia are rarely found on them, although their surface membranes are only in a few cases separated by glial processes. During the period of outgrowth of the dendritic tree (15 mm and older stages) most organelles of the soma are situated near the side of outgrowth, i.e. the apical part of the cell. The opposite, basal, part of the soma may still have an immature appearance with many free ribosomes (fig. 49, cf. Altman, '72b).

The distinction between the somata of Purkinje cells and eurydendroid cells could be made with certainty in 22 mm trout (in Golgi preparations this distinction can be made earlier). At this stage the somata of Purkinje cells have two rims of RER, one around the nucleus and the other one more peripherally under the cell membrane. Such concentrations of RER are absent in eurydendroid cells. These findings correspond to the lightmicroscopical observations on Nissl preparations. A young eurydendroid cell is shown in figure 50. Part of its soma still has an immature appearance. In older specimens (25 mm and

longer) a distinction between Purkinje cells and eurydendroid cells can be made on the basis of their afferent synapses.

In 22 mm trout glial processes enwrap parts of many Purkinje cells, a feature which is not observed in eurydendroid cells. It has been suggested by Altman ('72b) that the presence of a glial envelope is related to the presence of subsurface cisterns. Indeed, subsurface cisterns were encountered in the older Purkinje cells and not in eurydendroid cells. The assumption of Mugnaini ('69) that the development of subsurface cisterns precedes the formation of synapses, is not supported by my own observations. According to Le Beux ('72) in neurons subsurface cisterns are generally found opposite to glial cells, although a subsynaptic position may occasionally be observed.

The surface of Purkinje and eurydendroid cells in trout of about 20 to 30 mm is irregular due to the presence of perisomatic processes. Similar structures have been described for developing Purkinje cells in birds and mammals (Cajal, '11; Mugnaini, '69; Larramendi, '69; Altman, '72b), where they are more abundant than in the trout. The rather short and wide perisomatic processes contain either a dense network of very fine filaments or the same organelles as the perikaryon.

The dendrites of Purkinje cells contain, apart from the normally occurring organelles, subsurface cisterns and, in their proximal parts, ribosomes located near the surface membrane (figs. 49, 51). In older animals extremely long mitochondria, oriented parallel to the axis of the dendrites, are found.

In a number of dendritic growth cones very few vesicles and tubules of SER are present, their chief content consisting of a loose meshwork of fine filaments, as found for dendritic growth cones in mouse olfactory bulb (Hinds and Hinds, '72).

The shafts of developing Purkinje dendrites possess short protrusions and long and slender processes, the filopodia. Some of the latter are even observed in mature trout. The spines of tertiary and higher-order branches

appear in specimens of about 16 mm. These claviform structures contain some cisternae of SER and a fine filamentous network, which is slightly denser than in the dendritic shafts. Parallel fibres make definitive synapses with the spines, while in younger stages such fibres make transient synapses with the shafts of the dendrites.

The proximal dendrites of eurydendroid cells differ from proximal Purkinje dendrites in some respects: (1) their cytoplasm is less electron-dense, (2) ribosomes are evenly distributed, (3) subsurface cisterns are absent or very rare, (4) they contain fewer microtubules, which are less regularly arranged. Distal dendrites of eurydendroid cells bear only a few small spines. No attempt was made to distinguish them from other smooth dendrites in the molecular layer, *viz.* dendrites of Golgi cells and stellate cells. All of these structures are contacted by parallel fibres.

The developing molecular layer gradually attains a regular appearance. This feature may be linked up with the lightmicroscopical observations, that the dendritic trees of the Purkinje cells become gradually oriented in one plane.

As regards the *synaptology* of the neurons of the ganglionic layer, it has already been mentioned that the first synapses of the cerebellum occur between parallel fibres and the shafts of Purkinje dendrites. The first axosomatic synapses were observed in trout of 16 mm (5 days). The membrane specializations of these synapses are symmetrical. The presynaptic component contains elliptical vesicles, some mitochondria, SER and microtubules, and a denser filamentous network than the axodendritic synapses. Careful comparison with synaptic types occurring in later stages shows that these axosomatic synapses are made by axoncollaterals of Purkinje cells. As has been described above, Purkinje cells and eurydendroid cells still have the same ultrastructure in the 16 mm stage, hence the nature of the post-synaptic component could not be determined.

When the Purkinje dendrites start to form spines, synapses of parallel

fibres are found on these spines. Occasionally, synapses of the same type are present on the perikarya of the ganglionic layer. Such a junction is interpreted as a synapse of an ascending granule cell axon. In later stages parallel fibres almost exclusively contact the spines of Purkinje dendrites and only very rarely their shafts. No synapses with the Purkinje somata are found then.

In 17 mm trout, when there are still few axosomatic synapses, climbing fibre synapses are observed (fig. 51). They occur on small protrusions and on the smooth surface of the proximal dendrite of Purkinje cells. Presumably some are present on the somata. These synapses are characterized by asymmetrical membrane specializations and the occurrence of round, both clear and dense-cored vesicles, in the presynaptic component. Hitherto the latter structures were not found in other synapses. In the presynaptic bag some mitochondria, SER, microtubules and fine filaments are also present.

In 22 mm trout the number of parallel fibre synapses has considerably increased. The presynaptic component may be very large. The number of axosomatic synapses, formed by axoncollaterals of Purkinje cells, has only slightly increased. Synapses with the same characteristics are also found on the shafts of Purkinje dendrites.

During further development many new contacts are made by the axoncollaterals of Purkinje cells. It is possible now to distinguish between eurydendroid and Purkinje cells on the basis of the abundance of synapses on the soma and proximal dendrites of the former. The Purkinje cells have only few axosomatic synapses, which may be found on both the perisomatic processes and the smooth surface of the soma. It was not determined to which level in the molecular layer the Purkinje axoncollaterals extend. The presynaptic part of their synapses may contain some dense-cored vesicles now, but less than are found in climbing fibre terminals. The membrane specializations of these synapses show some variation. In the rare instances that they occur on spines they tend to be asymmetrical (Gray's type 1), but on smooth surfaces their pre- and post-

synaptic densities are of about the same width. The presynaptic densities may even extend further in the cytoplasm than the postsynaptic ones. My observations are in accordance with those of Mugnaini ('69, '70), who found that spines always form asymmetrical contacts.

The number of climbing fibre synapses does not increase very much; they are only occasionally found in the stages studied. In the adult they have not been observed so far, but a thorough analysis of the mature cerebellum might reveal their presence. According to my observations climbing fibre synapses occur preferentially on small protrusions of a soma or dendrite. The membrane specializations of climbing fibre synapses are always conspicuous and asymmetrical (Gray's type 1), although the post-synaptic densities are more conspicuous on knobs than on smooth surfaces (fig. 51).

Another type of synapse was observed on the dendritic shaft and soma of Purkinje cells. These synapses were found with certainty in 34 mm trout, but probably are present earlier. They have symmetrical membrane specializations. The presynaptic component is rather electron-translucent and contains flat vesicles, mitochondria and a few other organelles. These features correspond to those of synapses of stellate cell axons, as described in other vertebrates: e.g. frog (Sotelo, '70) and rat (Palay and Chan-Palay, '74), hence this type of synapse in the trout was interpreted as such.

2 b. *Neurons of the granular layer*

The radially oriented migrating cells in the molecular layer (fig. 47) are not essentially different from their tangentially oriented precursors. The cells appear to follow the glial processes which reach the meningeal surface with their terminals. The presence of radially arranged rows of granule cells in the granular layer may indicate that several granule cells follow the same migration path. In regions where bundles of ascending axons are already present, new-coming granule cells may migrate radially between these axons. Thus, for

their migration the cells are not dependent upon the presence of glial processes. In the ganglionic layer the migrating cells follow the surfaces of the glial and neuronal perikarya present there (fig. 50).

In granule cells that have reached the ganglionic layer, the cisternae of the RER start to lengthen. Conspicuous differentiation does not appear until the cells have reached their destination. In contrast with developing large neurons, neither their nuclei nor their perikarya show a distinct increase in size.

The extreme dark appearance of both nucleoplasm and cytoplasm disappears when a granule cell has settled in the granular layer. Its nucleus now attains a more or less round shape and contains irregular clumps of chromatin (fig. 53). The cytoplasm shows an increase in the amount of RER and a decrease in the number of free ribosomes, as has also been observed for the neurons of the ganglionic layer. In some cases an abundance of tubular and vesicular structures in connection with the Golgi complex is found, a feature which is probably related to dendritic outgrowth.

The axons of granule cells, both ascending and parallel fibres, contain microtubuli, some SER, mitochondria and a loose meshwork of very fine filaments, all of which is usual for axons, but very few neurofilaments. The dendrites of granule cells similarly show the usual characteristics of dendrites, but neurofilaments and microtubules are rather scarce.

Scanty information is available on the differentiation of Golgi cells. My observations suggest that their development proceeds in the same way as that of the other large neurons, i.e. the Purkinje and eurydendroid cells. However, Golgi cells are distinguished from the latter neurons by their slightly lobulated nucleus and by the configuration of their RER membranes. During development as well as in the adult these membranes together show a conspicuous reticulum.

Turning now to the *synaptology*, it appears that both granule and Golgi

cells participate in the formation of the glomeruli, the synaptic complexes of the granular layer. The central part of a glomerulus is formed by a mossy fibre rosette. Mossy-fibre synapses are observed for the first time in 17 mm trout, but true glomeruli are not formed as yet. The latter develop gradually and are clearly recognized in 34 mm trout. Figure 52 shows a synaptic region in the granular layer of an 82 mm trout. Mossy fibres contain more neurofilaments than other axons in the cerebellum and can easily be recognized. The rosette has a very irregular form (cf. fig. 27). The long filiform appendages, observed in Golgi preparations, were not identified with the electronmicroscope. In the rosette many round or nearly round synaptic vesicles, some dense-cored vesicles, SER and many mitochondria are found. The membrane specializations at the synaptic sites are conspicuous and asymmetrical. The postsynaptic structures are dendrites of granule cells. In the peripheral part of the glomerulus axonal endings are observed, which synapse with the granule cell dendrites. These endings contain ellipsoid vesicles. In accordance with my lightmicroscopical observations and with the data in the literature on the structure of the mammalian glomerulus, the endings are interpreted as axons of Golgi cells. The glomerulus is surrounded by glial processes. As lightmicroscopy has shown, these glial processes belong to velate astrocytes or astrocytoid ependymal cells.

Electronmicroscopy further revealed the following synaptic relations. The parallel fibres synapse with all the types of dendrites in the molecular layer and, in addition, with the somata of stellate cells. In a very small number of cases the ascending axon of a granule cell contacted a Purkinje dendrite. The somata of granule cells have never been observed to bear synapses.

The afferent connections of the Golgi cells are largely formed by the parallel fibres. A Golgi cell, the soma of which is situated close to the ganglionic layer, appears to receive axoncollaterals of Purkinje cells, as is found for Golgi cells in the mouse (Larramendi and Lemkey-Johnston, '70) and

the rat (Chan-Palay, '71). Scheibel and Scheibel ('54) and Chan-Palay ('71) discovered a number of other afferent relations in mammals; a thorough analysis would be needed to reveal their possible presence in the trout.

2 c. *Neurons of the molecular layer*

The electronmicroscopical observations are in accordance with the view that stellate cells originate from the secondary matrix. Rakic ('73) believes that a certain degree of maturation of the molecular layer is a prerequisite for the differentiation of stellate cells. My observations are in accordance with this view, since differentiating stellate cells are not recognized until the molecular layer has reached a certain thickness, i.e. from the 17 mm stage onward. The nucleus of stellate cells assumes a slightly irregular shape. The clear nucleoplasm lodges irregular clumps of chromatin, and in this respect the nuclei of stellate cells resemble granule cell nuclei. As in the latter, no increase of size is observed. The increase in the number of organelles, particularly of RER in the perikaryon, is less than in large neurons, but more than in granule cells.

Lightmicroscopy showed that the orientation of stellate cells is not perpendicular to that of the parallel fibres. Thus, the statement of Altman ('72a) that these elements on the basis of their orientation have to be considered as non-migratory, does not hold true for the trout. Moreover, stellate cells are also found in those regions where no secondary matrix layer has been present. It is hypothesized, therefore, that stellate cells are able to migrate over some distance first, and then, by the development of new processes in various regions, is rendered immobile. Young stellate cells have been found between bundles of parallel fibres, and oriented parallel to them.

The *synaptology* of the stellate cells has already been dealt with. Summarizing, it may be stated that the afferent connections are with parallel fibres, the efferent connections with the smooth dendritic surface and the

soma of Purkinje cells, as was found for stellate cells in the frog (Sotelo, '70).

3 *Neuroglial elements*

Electronmicroscopically, the following neuroglial elements could be identified: ependymal cells, astrocytoid ependymal cells, velate astrocytes and Golgi epithelial cells. The identification is based on their position and overall shape, since the ultrastructure of all these types proved to be essentially the same. Figure 53 shows some mature astrocytoid ependymal cells.

In 14 mm trout glial somata still resemble mantle cells, although their processes clearly could be recognized (fig. 46). Later, differentiation proceeds rapidly and glial cells with a mature appearance could already be found in 17 mm trout. The increase in organelles is less than in neurons and since the cells as a whole grow, the cytoplasm becomes more electron-translucent than that of neurons. Characteristic for glial perikarya is the presence of a number of parallel RER cisternae. Sometimes only a few rather short membranes were observed, but in other cases more and longer membranes, which even may be coiled up. The amount of SER is distinctly larger than in neurons. The shape of the nucleus is variable and seems to conform to the overall shape of the soma, which is variable as well: elongated cells have elongated nuclei and in the more rounded cells the nuclei are rounded too. Contrary to the observations in the toad (Stensaas and Stensaas, '68a, b), the monkey (Phillips, '73) and the rat (Ling *et al.*, '73), the nucleus of astroglial cells in the trout may have rather deep indentations. Variability of shape seems to be a general feature of glial cells (see e.g. Bleier, '71). The nuclear chromatin is either rather evenly distributed or concentrated in conspicuous clumps throughout the nucleoplasm and against the inner nuclear membrane. Such distributions were also found by Stensaas and Stensaas ('68) in the toad.

The characteristics of developing glial processes have already been

described. In the more distal parts of the processes the number of microtubules decreases. In some cases a number of RER cisternae was found at a considerable distance from the soma. In the submeningeal terminals, belonging to Bergmann fibres and processes of ependymoid astrocytes, parallel-running membranes of SER were found from about the 27 mm stage onward. A similar configuration of SER in the submeningeal terminals has been described for the rat (Palay and Chan-Palay, '74). In some cases wide, clear structures, resembling growth cones, were observed between the terminals, which touch the external limiting membrane. These are interpreted as the tip of a glial branch, reaching the external surface and starting to participate in the layer of terminals.

The submeningeal terminals are connected by junctions, varying from puncta adhaerentia to zonulae adhaerentes, and junctions with narrowed or obliterated extracellular space (gap junctions and tight junctions, respectively). The gap junctions and tight junctions occur over a considerable distance. Puncta adhaerentia are formed by all parts of the glial cells with their surrounding structures, either glial or neuronal, but more frequently glial.

My observations suggest that the amount of glycogen, often abundantly present in very young glial cells, decreases during development. Vaughn and Grieshaber ('72) described a decrease in the amount of glycogen in developing rat spinal cord.

The Bergmann fibres, which enclose the Purkinje dendrites in particular, have a very low organelle content. The glial investment of the dendrites on non-synaptic sites is completed after the formation of definitive synapses (cf. Altman, '72b, c). In trout of about 20 mm, only a small part of the Purkinje soma and dendritic tree is covered by glial processes, but the investment proceeds gradually and in the adult almost all of the non-synaptic surface of these elements is surrounded by glia.

A similar investment by glia is observed for the bloodvessels in the cerebellum. As has been described, bloodvessels enter the cerebellum in about

the 9 mm stage. I found that in 16 mm trout no astrocytic processes cover the endothelium. In the 17 mm stage some of these processes are present and in 82 mm trout they surround a large part of the bloodvessels. A comparable mode of investment of bloodvessels is described by Phelps ('72) in developing rat spinal cord.

In glia, the density of the very fine filamentous network of the cytoplasm, is somewhat variable. Generally, it is rather loosely meshed, but in the period of extensive glial investment of the target structures the density increases considerably.

Observations on smooth protoplasmic astrocytes and microglial cells have not been made. Oligodendrocytes were found occasionally. These elements have an irregularly-shaped nucleus with unevenly distributed, conspicuous clumps of chromatin. Their cytoplasm is very electron-dense. Parallel running membranes of RER are present and microtubules are numerous.

Discussion

As far as I know, electronmicroscopical observations on the earliest stages of cerebellar development have been described here for the first time. The ultrastructure of the matrix cells in the cerebellar anlage of the trout, appears to correspond closely to that of matrix cells in various regions of the brain in other vertebrates: chick spinal cord (Lyser, '64, '68a, '71), mouse cerebral vesicle (Hinds and Ruffett, '71), mouse olfactory bulb (Hinds, '72). In the regions mentioned, only the differentiation of neuroblasts has been studied; the development of glioblasts is left out of consideration. In the studies of Meller *et al.* ('66b), Meller and Haupt ('67) and Glees and Meller ('68) the precursors of both the neurons and the neuroglial elements are described. According to these authors, the first recognizable glioblasts are found at a time, when processes could not yet be identified. These elements would be characterized by a lobulated nucleus, the cytoplasm containing

less ribosomes and RER than that of neuroblasts. For the cerebellum of the trout these criteria appear not to be valid, since neuroblasts as well pass through a stage with a more or less lobulated nucleus and little RER (fig. 46, cell 3). Contrary to the findings of the above mentioned investigators, in young stages a distinction between neuroblasts and glioblasts can only be made on the basis of the structure of the processes (fig. 46). In later stages, neuronal nuclei tend to be round, whereas the nuclear shape of glial cells is variable. An increase in the amount of RER is observed in both neurons and glial cells, although generally to a lesser degree in the latter.

According to my observations, the endoplasmic reticulum membranes develop from two different sources. In the early stages the Golgi complex produces tubular smooth profiles, to which ribosomes become attached. In later stages, when most of the RER is formed, the remainder of this organelle develops from the outer nuclear membrane. According to Meller *et al.* ('66b) the Golgi complex provides most of the ER membranes, whereas Pannese ('74) believes that the outer nuclear membrane is the sole source. It is beyond the scope of the present study to trace the origin of all of the cytoplasmic organelles or to discuss their possible functions. For a discussion of these aspects, the reader is referred to the work of Pannese ('74).

As regards the development of the cerebellar cell types, the lightmicroscopical observations are confirmed and amplified. It seems to be a general principle that the morphological identification of cell types can be made earlier on the basis of the structure of their processes than on the basis of the structure of their somata. This was shown for neuroblasts and glioblasts and also holds true for Purkinje cells, eurydendroid cells and granule cells.

The perisomatic processes of the developing neurons of the ganglionic layer, are generally wider than filopodia, but have a similar content of fine filamentous material. In some cases however, they contain the usual cytoplasmic organelles. It is hypothesized, that these processes, when being formed, con-

tain only the fine filaments as in filopodia, the next step being a penetration of organelles. The last phase would be a smoothening of the surface by growth of the whole cell. According to this view, the presence of perisomatic processes on cells is related to growth. The same opinion has been expressed by Mugnaini ('69). In the trout perisomatic processes are not very numerous. Synapses are found on these structures, but since they occur on the smooth surface of the soma as well, a clear correlation in the occurrence of synapses and perisomatic processes could not be demonstrated. Larramendi ('69) and Altman ('72b) believe that the presence of perisomatic processes on Purkinje cells is related to the "phase du nid" (Cajal, '11) of developing climbing fibres.

Protrusions and spines on the developing Purkinje dendrites are smaller and shorter than filopodia but contain a similar, fine filamentous network. In addition, some SER may be present. SER is always present in the permanent structures, i.e. the clavate spines.

The migration of the derivatives of the secondary matrix layer, particularly the granule cells, deserves some comment. Berry and Rogers ('65) and Morest ('70), studying the development of the mammalian cerebral cortex, concluded that migration of neuroblasts as a whole does not occur, translocation of the nucleus within the processes of the cell giving the false impression of migration. Rakic, on the other hand, studying the developing cerebral ('71, '72) and cerebellar ('71) cortex, arrived at a different opinion. Mugnaini and Forstrønen ('67) and Rakic ('70) had noticed already that in the cerebellum migrating granule cells and Bergmann fibres are frequently lying in apposition. In the view of Rakic, migration of neuroblasts takes place, not "freely" but guided by the radially-oriented glial processes to which the neuroblasts are apposed. Moreover, Weaver mutant mice, in which most of the future granule cells die shortly after birth, appear to have hardly any Bergmann fibres and it is assumed that the death of the cells is caused by the lack of the substrate

along which they normally migrate (Rakic and Sidman, '73a, b). In my opinion such a direct causal relationship is not necessarily present, however.

As for the trout, the Rakic-hypothesis was clearly confirmed. Yet, Bergmann fibres are not the sole structures along which the young granule cells migrate. The first migrating cells in the molecular layer presumably all descend along glial fibres, *viz.* Bergmann fibres and the processes of ependymoid astrocytes, but elements migrating later may follow the bundles of ascending granule cell axons, which are then present. In the ganglionic layer migration occurs along its constituent neurons and glial cells. Comparable observations have been made by Das *et al.* ('74).

The tangential migration of secondary matrix cells seems more difficult to understand. However, as in the case of radial migration, glial cells may play a role. The upper layer of secondary matrix cells is always in contact with the membrana limitans gliae. Lower rows of cells, if present, seem to follow the elements of a higher level. Egar and Singer ('72), studying regeneration of Triturus spinal cord, found that the radially-arranged ependymal processes enclose rostrocaudally-directed tunnel-like pathways, in which small, unmyelinated axons are present. These authors conjecture, that the ependyma forms a pathway to guide regenerating fibres caudally. Apart from glial cells other structures may play a role in tangential migration in the cerebellum of the trout. In stages when bundles of parallel fibres have been formed, secondary matrix cells may follow these bundles.

The factors regulating onset, continuation and end of migration are completely unknown. The electronmicroscopical observations support the hypothesis, presented in the previous chapter, that an accompanying feature of migration is the presence of a dense filamentous network in the migrating cell. The phenomenon "dark cell" is discussed in the next chapter.

Although sites of specialized contact are present in all stages, synapses are observed for the first time in hatchlings. The development of the para-

membranous specializations of synapses was not analyzed in the present study (see Aghajanian and Bloom, '67 and Adinolfi, '72a, b, for this aspect of synaptogenesis), nor was the origin of synaptic vesicles. Of the various theories on the latter problem, we mention an origin from neurotubules (Palay, '58), from the Golgi apparatus and SER (Stelzner, '71), by invagination of the presynaptic membrane (Westrum, '65; Turner and Harris, '73).

According to Altman ('71, '72b) and Stelzner *et al.* ('73) the presence of coated vesicles is associated with synaptogenesis. Privat ('74), however, considers coated vesicles as resorbed puncta adhaerentia. Coated vesicles were only occasionally encountered in the material of the trout and a relation with synaptogenesis could not be demonstrated.

Most of the investigators studying synaptogenesis agree that the first-appearing synapses are axodendritic (as for the cerebellum: del Cerro and Snider, '72; Foelix and Oppenheim, '74, the present study). Foelix and Oppenheim ('74), consider these axodendritic synapses in the chick to be made by climbing fibres. The first synapses in the cerebellum of the trout resemble the synapses of mature parallel fibres rather than the earliest synapses of climbing fibres, and, hence, I interpret them as contacts between parallel fibres and young Purkinje dendrites. However, the presynaptic component of the first axodendritic synapses in the chick (Foelix and Oppenheim, '74) contains dense-cored vesicles, which are absent in the first synapses but present in the climbing fibre synapses of the trout. Del Cerro and Snider ('72), who did not attempt to classify the earliest appearing synapses in the cerebellum of the rat, mention the occurrence of dense-cored vesicles in the presynaptic component as well. The interpretation of Foelix and Oppenheim is at variance with several other studies, all of which have shown that climbing fibres contact the somata of Purkinje cells first (Cajal, '11; Larramendi, '69; O'Leary *et al.*, '71; Kornguth and Scott, '72; Altman, '72b).

Young synapses in the trout sufficiently resemble the mature structures

so as to permit classification on the basis of similar criteria.

The sequence in which the synaptic types appear in the trout, is as follows (only the presynaptic component is mentioned): (1) parallel fibre, (2) Purkinje cell axon, (3) climbing fibre and mossy fibre, (4) stellate cell axon. Parallel fibres contact the shafts of the Purkinje dendrites in young stages, establishing permanent synapses with the spines of tertiary and higher-order dendritic branches later on. Similar transient and permanent synapses have been described for Purkinje cells in the chick (Mugnaini, '69) and in the rat (Altman, '72b).

The morphology and the physiology of synapses have been correlated by Uchizono ('65, '75). According to him excitatory synapses contain round vesicles, whereas inhibitory synapses contain ellipsoid (flat) vesicles. Applied to the cerebellum of the trout, this would mean that mossy fibres, climbing fibres and granule cells exert an excitatory action, whereas Purkinje cells, Golgi cells and stellate cells are inhibitory. These assumptions are in agreement with the findings obtained in physiological work on the trout (Waks, '71) and on other vertebrates (e.g. Eccles *et al.*, '67). The difference in shape of synaptic vesicles in young stages is slightly less pronounced than in the adult. This feature may be due to a different reaction to the fixation fluid (Valdivia, '70).

As regards the circuitry of the teleostean cerebellum, it is generally believed that the afferent systems terminate as mossy and climbing fibres, and that the axons of the Purkinje cells constitute the efferent connections (Ariëns Kappers *et al.*, '36; Larsell, '67). In addition, a nucleus cerebelli (comparable to the deep cerebellar nuclei of mammals) has been described, which receives the axons of a number of Purkinje cells, and whose axons leave the cerebellum (Pearson, '36). Nieuwenhuys and Nicholson ('69) and Nieuwenhuys *et al.* ('74) described the eurydendroid cells in the ganglionic layer of the mormyrid cerebellum and suggested that these elements constitute the output system,

in this respect being comparable to the cells of the deep cerebellar nuclei of higher vertebrates. The present study showed that eurydendroid cells are also present in the trout. They receive axon collaterals of Purkinje cells and in a few cases it was observed that their axons leave the cerebellum. The probable neuronal circuits in the adult trout are schematically represented in figure 54. The main input - output system is formed by the mossy fibres, the granule cells, the Purkinje cells and the eurydendroid cells. The shortest path, i.e. the path with the fewest synaptic interruptions, is formed by the mossy fibres, granule cells and eurydendroid cells, or, starting from the other type of afferents, the climbing fibres, Purkinje cells and eurydendroid cells. These afferent-efferent systems are influenced by the following internuntial elements. (1) The Golgi cells. They receive their input from parallel fibres and synapse with the dendrites of granule cells, which they are supposed to inhibit. (2) Axoncollaterals of Purkinje cells, exerting an inhibitory action on Golgi cells. (3) Axoncollaterals of Purkinje cells, contacting other Purkinje cells. The inhibition of the latter elements results in a disinhibition of the eurydendroid cells contacted by them. (4) The inhibitory stellate cells, synapsing with Purkinje cells, and thus exerting an indirect facilitatory action on the eurydendroid cells. The physiological influence of eurydendroid cells is unknown. However, if these elements are comparable to the cells of the deep cerebellar nuclei of other vertebrates, they are probably excitatory.

Although a nucleus cerebelli as described by Pearson ('36) has been found in the present study, it is left out of consideration in the discussion on the circuitry of the cerebellum. The number of cells in this nucleus is very small compared with the number of Purkinje cells. It is possible, however, that the axons of some Purkinje cells terminate in this nucleus.

Climbing fibres were not found in the mature cerebellum so far, but Waks ('71), studying the same species, claimed to have observed them. The electrophysiological experiments of Waks ('71) and Peterson ('72) have lent further

support to the view that climbing fibres are present in the mature teleostean cerebellum. Concerning the origin of climbing fibres, there is no unanimity. The original concept, that the inferior olive is the only source of climbing fibres, has been challenged by a number of authors of whom we mention Llinás *et al.* ('67), Sasaki ('69), O'Leary *et al.* ('70), Rivera-Dominguez *et al.* ('73), Batini *et al.* ('73) and Destombes ('73). However, in a recent paper, Desclin ('74) holds that in mammals the inferior olive is the major and probably sole significant source of climbing fibres. In teleosts, a small inferior olive has been described (Kooy, '17; Ariëns Kappers *et al.*, '36; Kremers, '74, unpublished observations). If this structure is the principal source of climbing fibres, the number of these fibres in the cerebellum cannot be very large. It seems probable that mossy fibres provide the principal input to the cerebellar cortex of the trout.

The development of the neuronal circuits does not follow a retrograde sequence compared to the actual flow of action potentials, as suggested by Larramendi ('69). Nor is the establishment of synaptic contacts started by the extrinsic fibres as Mugnaini ('69) asserted. Mossy and climbing fibres enter the cerebellum when many synapses have already been formed between parallel fibres and Purkinje dendrites. Synapses of Purkinje axons and their collaterals are present in an early stage, although the largest number of these synapses is formed rather late in development. Larramendi ('69) suggested that neurons are synaptically competent during certain periods, their surfaces having variable synaptogenetic capacities. A certain degree of differentiation is required before the cells are able to establish synapses. Applying Larramendi's concept to the cerebellum of the trout, it would follow that Purkinje and eurydendroid cells are synaptically competent during a long period. Also, it would follow that the synaptic capacities of the shafts of the Purkinje dendrites differ from that of the spines. As soon as dendritic spines have been formed, the shafts do not form synapses with parallel fibres

anymore, although these two structures may lie in close apposition. However, the axons of Purkinje cells and stellate cells are at that time still capable of establishing synapses with the shafts. The somata of Purkinje cells bear only few synapses and are enveloped by glial processes rather soon. Hence, synaptogenesis comes to an end (Altman, '72b, c). It is unknown whether a relation exists between glial covering and synaptic competence.

With regard to the origin of the neuroglial cells, electronmicroscopy amplified the view that most of these elements are derivatives of the ventricular matrix layer. From his autoradiographic studies on the cerebellum of chick and mouse, Fujita ('69) concluded that the ventricular matrix commences glioblast formation when the production of neuroblasts has come to an end. Glioblasts are considered to be "free", i.e. not attached to either the ventricular or meningeal surface. Fujita observed them for the first time when the external matrix layer had reached its maximal thickness, but inward migration of future granule cells did not yet occur. According to Das *et al.* ('74), Golgi epithelial cells in the rat are predominantly formed between 9 and 12 days postnatally, i.e. when a considerable number of granule cells has already reached the granular layer (Altman, '72c). The layer of submeningeal glial terminals is generally believed to be formed after birth in mammals (del Cerro and Snider, '72; Altman, '72a, '75), or in late developmental stages in the chick (Mugnaini and Forstrønen, '67). The present study showed that in the cerebellum of the trout the precursors of Golgi epithelial cells are attached to the meningeal surface from the earliest stages on (cf. Cajal, '11). Glial differentiation was observed already in 7 mm embryos. I conjecture that a number of immature Golgi epithelial cells are also present in embryonic stages of mammals, in spite of the data from literature mentioned above. In favour of this view are the following data. Rakic ('71) shows a very immature Golgi epithelial cell, that closely resembles a matrix cell (cell a in his figures 1 and 6). Das *et al.* ('74) described the presence of "thin gray-looking

processes of unknown origin" in the molecular layer of the rat, occurring prior to the period in which the Golgi epithelial cells are considered to be born. Cells from the external matrix layer were observed to migrate along these processes. The postnatal formation of Golgi epithelial cells, as found by these authors, can be explained by assuming a considerable proliferation of glioblasts during the postnatal expansion of the cerebellum.

This chapter will be devoted to the following general problems: (1) The homogeneity of the matrix layer, (2) The occurrence of mitotic figures in the matrix and the mantle layer, (3) The relation between the phenomena growth and migration, and the occurrence of fine filaments, (4) The relation between the occurrence of cell junctions and differentiation.

The homogeneity of the matrix layer

Schaper (1894, '97) was the first who understood that the spongioblasts and germinal cells of His (1889), constituting the early neural tube, are not two different types of cells, one giving rise to glioblasts and the other to neuroblasts, but rather a single type of epithelial cell in different stages of the mitotic cycle. Schapers opinion was confirmed by Sauer ('35) who gave a detailed description of the mitotic cycle of these epithelial cells. Fujita ('62, '63) termed the elements in question matrix cells. Since these cells have the capacity to divide, as well as to produce both neuronal and glial precursors, the term matrix is appropriate and has been used throughout the present study. Electronmicroscopical studies (Lyser, '64, '68a; Hinds and Ruffett, '71 among others) showed the structural homogeneity of the population of matrix cells at the ultrastructural level.

The homogeneity of the matrix layer as a whole is a matter of discussion in the literature. Are only matrix cells involved or are other cell types present among these elements? The last possibility was favoured by Schaper (1897), who described within the matrix layer (his layer of "primitive Epithelzellen") of reptiles, birds and mammals, the formation of so-called indifferent cells, destined to differentiate into the neuroblasts and glioblasts of the mantle layer. According to Schaper, in lower vertebrates the indifferent cells differentiate into neuroblasts and glioblasts already within the matrix layer. Amphioxus would be the only animal producing neuroblasts and glioblasts direct-

ly from "epithelial" cells. Indifferent cells have not been observed by Cajal ('08), who described the development of neuroblasts within the matrix layer in the chick. Cajal utilized silver impregnation techniques. According to him, neuroblasts are recognizable by the presence of neurofibrils. These observations were confirmed by a number of investigators, among whom Windle and co-workers ('36a, b) and Barron ('43, '46), studying some mammalian species and the chick. The genesis of glioblasts is left out of consideration in these studies.

Fujita ('63) arrived at the conclusion that no other elements than matrix cells compose the matrix layer. Using the technique of cumulative labeling of cells with ^{3}H-thymidine, he found that the percentage of labeled matrix cells increased linearly with time and reached 100%. In his words: "This finding indicates that the matrix cells in the neural tube form a functionally homogeneous cell population and that the matrix layer is composed exclusively of the matrix cells". Also, in his opinion, cytodifferentiation "is determined within the matrix layer while the undifferentiated cell is in the postmitotic resting period t_1. The cells so destined to differentiate leave the matrix layer very rapidly and their cytodifferentiation becomes evident after they leave it. "Neurons, neuroglia and ependymal cells are considered to derive directly from the matrix cells at successive stages of development (Fujita, '63, '65, '69).

Lyser ('64, '68), studying electronmicroscopically the development of neurons in the chick spinal cord, described early neuroblasts within the matrix layer as cells provided with axons. She was unable to find a clear correlate of the neurofibrils observed in lightmicroscopy, although some microfilaments appeared to be present in presumptive neuroblasts. Sechrist ('68, '69) on the other hand, claimed that neuroblasts can be identified even prior to their last mitosis on the basis of conspicuous aggregates of 60 Å filaments. These structures would represent the argentophilic neurofibrils observed with

lightmicroscopy. Sechrist used autoradiographic and silver-staining techniques in combination with electronmicroscopy. Distinct argentophilia could be demonstrated in developing chick retina only, the other species studied (axolotl, hamster) reacting negatively or just faintly positively with the silver impregnations. By assuming that neuroblasts are recognizable prior to their last mitosis and leave the matrix layer after their last division, Sechrist claimed to have cleared up the controversy between the theories of Cajal and Fujita.

A few years later, Hinds and Ruffett ('71) challenged the validity of Sechrist's statements. Studying electronmicroscopically the mitotic cycle of matrix cells in the cerebral vesicle of the mouse, these authors observed 60 Å filaments in all phases of mitosis, some cells showing even large accumulations of such filaments. They concluded that "these accumulations of microfilaments may be non-specific, perhaps related to the outgrowth of the external process of the ventricular cell". They further suggested that some apolar and bipolar neuroblasts, described by Cajal on the basis of silver impregnation, might be undifferentiated matrix cells in mitosis or interphase. According to Hinds and Ruffett it is well possible that the determination of a matrix cell to become a neuroblast occurs only after the outgrowth of its external process into the mantle layer, where there is a chance to interact with the differentiating neurons and afferent axons. In this view, neuroblasts may have internal and external processes just as matrix cells, as has been described for neuroblasts in the developing forebrain of the opossum (Morest, '70). However, according to Cajal ('08), Lyser ('64, '68) and Sechrist ('68, '69) the axon of neuroblasts is comparable to the external process of matrix cells.

My observations on the early cerebellar anlage of the trout will be discussed against the background of the opinions mentioned above. In the Bodian series of the early stages the cerebellar anlage showed no argentophilia. This may be due either to the possibility that the staining technique was sub-

optimal for demonstrating argentophilic structures, or to the absence of argentophilia. Argentophilia is not necessarily a property of a special structure, for instance neurofibrils, but depends in the first place on an appropriate redox potential (Wolman, '55; Peters, '55). The redox potential of the structures to be shown may vary between different species, as well as alter during the development of one species. My observations do not contradict the opinion of Sechrist ('68, '69), that accumulations of microfilaments are the structures responsible for silver staining, since such accumulations are not present in the matrix and the mantle cells of the cerebellum of the trout. The absence of these structures is not due to the fixation procedure, since filaments are observed in early and later stages with the same fixation technique. The cytoplasm of "dark cells", in which a dense filamentous network is present, is not argentophilic. Thus, in the trout, neuroblasts cannot be distinguished on the basis of accumulations of neurofibrils (with the light-microscope) or neurofilaments (with the electronmicroscope) (cf. Cajal, '08; Sechrist, '68, '69), irrespective of the consideration if it would be correct to do so (cf. Hinds and Ruffett, '71). So far, I have not been able to determine whether neuroblasts are present between the matrix cells. Axonal profiles are found in the matrix layer as soon as a mantle layer is formed, but it might be possible that these axons belong to cells, the nucleus of which is already located in the mantle layer (cf. Morest, '70). The hypothesis of Hinds and Ruffett ('71) that determination of a matrix cell as a neuroblast occurs after outgrowth of the external process into the mantle layer, by interaction with differentiating neurons and afferent axons, is not valid for the trout. Afferent axons are not yet found in the early cerebellar anlage, hence the hypothesis does not explain the appearance of the first neuroblasts.

In my opinion the following observations render it probable that glioblasts are present in the matrix layer. (1) In late-embryonic stages the cells, lining the ventricle at the level of the lateral thickenings, start to

differentiate. At least part of these cells develop into astrocytoid ependymal cells, as was shown by Golgi preparations and electronmicroscopical preparations of young trout. (2) In those regions where a matrix layer persists, glial elements are found among the matrix cells. It is concluded that a number of glial cells do not migrate but differentiate *in situ*. In young-embryonic stages glial differentiation manifests itself only in the peripheral processes of the cells. Considering the observations just mentioned, it is well possible that at least part of the glial somata, belonging to these processes, occupy a position in the matrix layer. However, these young glioblasts are indistinguishable from matrix cells. Stensaas and Stensaas ('68d) and Leonhardt ('72), studying the matrixzone in the telencephalon of late-embryonic and adult rabbits, respectively, were able to distinguish both neuroblasts and glioblasts in that zone. If the results obtained in mammals with autoradiography (Fujita, '63), that the percentage of labeled cells in the matrix layer increases linearly with time, were confirmed for the trout, it would have to be assumed that glioblasts in the matrix layer of that species still participate in the mitotic cycle. This is a reasonable assumption, since it is well known that glioblasts and even glial cells are capable of proliferation (see Jacobson, '70).

The occurrence of mitotic figures in the matrix and the mantle layer

Early histologists (e.g. His, 1889; Schaper, 1894, '97) already noticed that the ventricular surface is the preferential site for a matrix cell to divide. These observations have been confirmed by Sauer ('35), who described the mitotic cycle of matrix cells in detail. The occurrence of mitoses peripheral to the ventricular surface was quantitatively studied in some regions of mouse cerebrum and spinal cord by Smart ('72a, b, '73). That investigator observed that non-ventricular mitoses occurred in those areas where the ventricular surface was completely occupied by dividing cells and concluded that lack of space at the surface forces the cells to divide peripherally. However,

since in the mouse diencephalon and cerebral hemisphere non-surface mitoses were predominantly found at the outer boundary of the matrix layer and had their cleaving plane parallel to the ventricular surface, Smart realized that some controlling mechanism must operate as well.

In the cerebellar anlage of the trout non-ventricular mitoses are observed, although the ventricular surface is never completely occupied by dividing cells. Yet, the highest number of peripheral mitoses is found when the proliferative rate at the ventricular surface is maximal. A relation between these two phenomena is probable, although lack of space at the surface does not seem to play a role. As has been found by Smart in the mouse, many peripheral mitoses are situated in the outer zone of the matrix layer and the plane of division of most of these mitoses is parallel to the surface. It seems unlikely, that both daughter cells of a cell dividing parallel to the surface return to the mitotic cycle of the matrix layer; one or both of them will probably migrate to the mantle layer. A number of peripheral mitoses is found in the mantle layer, most of them occurring in the period of highest overall mitotic activity. These mitotic cells may be either matrix cells, the nucleus of which has migrated to a position in the mantle layer, or glioblasts. If we assume that the determination of neuroblasts takes place prior to their last mitosis (Sechrist, '68, '69), there is also the possibility that the dividing cells are neuroblasts. Studying the brain of three-day old mice with autoradiographic techniques, Smart ('65) concluded that peripheral neuroblast mitosis does occur.

The secondary matrix layer of the cerebellum shows that the ventricular surface is not the only preferential site for mitosis. A large number of secondary matrix cells occupy a position subjacent to the membrana limitans gliae. I was unable to follow the differentiation of these cells into neuroblasts and glioblasts. Mitotic cells with the characteristics of secondary matrix cells are even found in regions which they must have reached after

a considerable radial migration. However, most of the migrating cells in these regions are to be considered as primordial granule cells.

The relation between the phenomena growth and migration and the occurrence of fine filaments

The present study showed, that growth cones and filopodia are characteristic of most and possibly all growing cell types, ranging from matrix cells to neurons and glial cells. The ultrastructure of growth cones in the cerebellum of the trout corresponds to that of growth cones in the mammalian cerebellum (del Cerro and Snider, '68; Kawana *et al.*, '71). They contain a loose filamentous network and variable numbers of cytoplasmic organelles, particularly SER. In filopodia generally only a dense network of fine filaments is found. The latter is also present in "dark cells", which I consider to be migratory. Growth and migration have in common the phenomenon motility. The relation between motility and the presence of filaments has been studied in a wide variety of biological systems (references in the papers of Hinds and Hinds, '72 and Kawana *et al.*, '71). The work of Wessells and co-workers is of particular interest (Wessells *et al.*, '71; Spooner *et al.*, '71; Yamada *et al.*, '71). These investigators studied the effect of the drug cytochalasin B on several processes *in vivo* and *in vitro* in animal and plant cells. The drug selectively blocked growth and movement of cells in which a filamentous network had been demonstrated. It appeared that the latter was disrupted by the drug. No other effects were found.

One additional comment on the occurrence of fine filaments should be made. It should be kept in mind that fixed tissues are examined, in which the proteins of the cytoplasm are possibly precipitated in the form of a network of fine filaments. It has been mentioned that a few filaments may even be found in the extracellular space. Evidence is overwhelming that a dense network of filaments is coupled with motility. However, it would be incorrect to

ascribe a function to all of the filaments observed in electronmicroscopy.

The relation between the occurrence of non-synaptic cell junctions and differentiation

The distribution of non-synaptic cell junctions appeared to change during the development of the cerebellum of the trout. In general, junctional complexes are most numerous between matrix cells, their number increasing with age. On the other hand, developing neurons show a strong decrease in the number of junctions with each other and with other surrounding structures. Developing glial cells tend to retain the number of junctions originally present. They have in particular junctions with each other, but with other structures as well. Loewenstein ('68a, b) supposed that a relation exists between the occurrence of junctions and cell differentiation. From his experiments and theoretical considerations he concluded that "there is a reasonably good chance that junctional communication is instrumental in the flow of signals controlling gene activity concerned with growth and differentiation", and that "both establishment (coupling) and interruption of signal flow (uncoupling) may give rise to differentiation under certain conditions" (Loewenstein, '68b, p. 171). Applying this hypothesis to the developing cerebellum of the trout, uncoupling would evoke differentiation of the matrix cells into neurons, while maintenance of junctions would evoke the differentiation into glial cells. Recently, Rubin and Everhart ('73) showed that in ovary cells of the chinese hamster intercellular contact is required for the full expression of the morphological changes that are associated with the mitotic cycle. Differentiation is not involved here, but the importance of intercellular communication with respect to certain capacities of cells is clearly demonstrated.

The morphogenesis and histogenesis of the cerebellum of Salmo gairdneri RICHARDSON, 1836 were studied in fish ranging in length from 4.5 mm to 230 mm. For lightmicroscopy sagittal, transversal and horizontal series were stained with haematoxilin-eosin and according to Bodian, Nissl, Klüver-Barrera and Golgi. For electronmicroscopy routine techniques were used.

The morphogenesis of the three main parts of the cerebellum, i.e. corpus cerebelli, valvula cerebelli and lobus vestibulolateralis, can be summarized as follows. The early cerebellar anlage is a simple, transversely oriented plate, which is delimited from the tectum mesencephali by the fissura rhombomesencephalica. The corpus cerebelli develops from the dorsocaudal part of this plate, which grows caudally, arching over the fourth ventricle. The middle parts of the bilaterally symmetrical anlage of the corpus expand in a ventromedial direction and finally fuse in the median plane. The valvula cerebelli develops from the ventrorostral part of the cerebellar anlage, which grows rostrally into the ventricle of the midbrain. During this expansion the valvula is thrown into folds. The lobus vestibulolateralis is formed rostrally to the velum medullare posterius and the lateral recesses of the fourth ventricle.

The histogenesis may be divided in a period of early development and a period of later development. The early development is characterized by the formation of the three cerebellar layers, the later development by growth. In the early histogenesis two phases were distinguished. During the first phase the matrix layer produces the mantle layer, except in the paramedian region (matrixzone M). In regions where the mantle layer is formed, the matrix no longer occupies the whole width of the wall and is termed the ventricular matrix. The largest part of the ventricular matrix is gradually exhausted.

However, in some places this matrix persists as a layer of proliferating cells. This holds for the matrix surrounding the lateral recesses (matrixzone L) and the velum medullare posterius (matrixzone P). The second phase of histogenesis is characterized by the formation of a secondary matrix. Newly produced cells of the matrixzones M, L and P migrate away from their sites of origin towards the regions where a mantle layer has been formed previously. These migrating cells are termed secondary matrix cells. Migration of the cells produced during the first phase of histogenesis occurs in a radial direction. The direction of migration of the cells produced during the second phase is variable, namely, either radial, or tangential, or tangential followed by radial. The study of the histogenesis during early and later development yielded the following results:

(1) The neuroblasts produced in the first phase of histogenesis differentiate into the large neurons of the cerebellum: Purkinje cells, eurydendroid cells and Golgi cells; those produced in the second phase differentiate into the small neurons: granule cells and stellate cells.

(2) Most neuroglial elements derive from the ventricular matrix layer. All of these elements show a similar ultrastructure.

(3) The lobus vestibulolateralis is a derivative of the matrixzones L and P.

(4) The nucleus cerebelli, as described by Pearson ('36), originates from the ventricular matrix surrounding the lateral angle of the ventricle in the isthmus region.

(5) The morphological identification of the cell types can sooner be made on the basis of the structure of their processes than on the basis of the structure of their somata.

(6) Growth cones and filopodia are characteristic of most and possibly all growing cells.

(7) Migration of cells generally occurs along glial processes, although an

apposition to other structures may be observed as well. In ultra-thin and semi-thin sections immature migrating cells show a dark appearance, due to the presence of a dense filamentous network and of large amounts of free ribosomes in their cytoplasm.

(8) The first synapses are axodendritic contacts; axosomatic synapses appear somewhat later. The sequence in which the various synapses appear is as follows (only the presynaptic component is mentioned): parallel fibre, Purkinje cell axon, climbing fibre and mossy fibre, stellate cell axon.

(9) As regards the circuitry in the corpus cerebelli, mossy and climbing fibres are the afferents. The mossy fibres enter into synaptic contact with the granule cells; the climbing fibres presumably with the main dendritic branches of the Purkinje cells. The axons of the granule cells enter the molecular layer and synapse with the dendrites of all of the neuronal elements present in the corpus cerebelli. The eurydendroid cells, receiving the axoncollaterals of Purkinje cells, are considered to form the output-system. These afferent-efferent systems are influenced by a number of superimposed circuits.

The results are discussed in light of current neuroembryological views and concepts.

Een onderzoek werd ingesteld naar de morphogenese en de histogenese, zowel op lichtmicroscopisch als electronenmicroscopisch niveau, van het cerebellum van de regenboogforel, Salmo gairdneri RICHARDSON, 1836. Forellen die varieerden in lengte van 4.5 mm tot 230 mm werden gebruikt. Ten behoeve van de lichtmicroscopie werden sagittaal, transversaal en horizontaal gesneden series gekleurd met haematoxiline-eosine en volgens Bodian, Nissl en Klüver-Barrera. Verder werden van elk ontwikkelingsstadium enkele exemplaren geimpregneerd volgens de Golgi-techniek (diverse modificaties). Om nader inzicht in de morphogenese te verkrijgen, werden met behulp van haematoxiline-eosine series van een aantal stadia drie-dimensionele reconstructies van het cerebellum en omgevende structuren vervaardigd. Voor electronenmicroscopisch onderzoek werd van standaard-technieken gebruik gemaakt (fixatie in fosfaatgebufferde glutaaraldehyde en osmiumtetroxide, kleuring met uranylacetaat en loodcitraat). Semidunne (1-3 μ) epon coupes, gekleurd met toluidine-blauw of ongekleurd, vergemakkelijkten de overgang van licht- naar electronenmicroscopie.

Ter algemene orientatie werd eerst een korte beschrijving gegeven van het cerebellum van de volwassen forel (hoofdstuk III). Aan dit cerebellum kunnen de volgende delen worden onderscheiden (fig. 1, 2):

a) een sterk ontwikkeld, massief *corpus cerebelli*, dat de vierde ventrikel overwelft,

b) de *valvula cerebelli*, een relatief dunwandige structuur die zich onder het tectum mesencephali uitbreidt, en

c) een transversaal georienteerde structuur, de *lobus vestibulolateralis*. De caudale begrenzing van deze lobus wordt gevormd door het velum medullare posterius en de recessus laterales van de vierde ventrikel. De rostrolaterale

delen van deze lobus staan bekend als eminentiae gramulares.

Wat de morphogenese betreft (hoofdstuk IV) heeft het onderzoek de volgende resultaten opgeleverd (fig. 3-5, 7, 10):

1. Door laterale uitgroei van die delen van de vroeg-embryonale neurale buis die juist rostraal en caudaal van het latere isthmus-gebied gelegen zijn, ontstaat een fissuur, de fissura rhombo-mesencephalica. De caudale begrenzing van deze groeve, een transversaal gerichte plaat, is de cerebellum-aanleg.
2. De grens tussen corpus en valvula cerebelli wordt in laat-embryonale stadia door een transversaal verlopende groeve gemarkeerd.
3. De ontwikkeling van het corpus cerebelli verloopt als volgt:

a) de dorsocaudale rand (het velum medullare posterius) van de aanvankelijk plaatvormige cerebellum-aanleg groeit uit naar ventraal, waardoor het corpus koepelvormig wordt. Vervolgens gaat het corpus zich in caudale richting uitbreiden, terwijl het velum medullare posterius niet van plaats verandert. In latere stadia overwelft het corpus het grootste deel van de vierde ventrikel.

b) de middengedeelten van de bilateraal-symmetrische aanleg van het corpus breiden zich sterk uit in ventromediale richting en vergroeien tenslotte in het mediane vlak. Op deze wijze wordt het corpus een massieve structuur.
De onder a) en b) geschetste ontwikkelingsprocessen zijn reeds uitvoerig door Schaper (1894a, b) beschreven.

4. De valvula cerebelli vormt, gedurende haar uitgroei onder het tectum mesencephali, een groot aantal transversaal georienteerde plooien. De nieuw gevormde delen vergroeien niet met het tegmentum.
5. De vóór de recessus laterales van de vierde ventrikel gelegen delen van het cerebellum gaan zich reeds in een jong-embryonaal stadium in rostrolaterale richting verdikken. De verdikkingen zullen een onderdeel vormen van de lobus vestibulolateralis. Van alle delen die tot deze lobus behoren ontwik-

kelen zich de eminentiae granulares het laatst. Op het ontstaan van de lobus vestibulolateralis wordt in punt 6 van de histogenese nader ingegaan.

De resultaten van het onderzoek naar de histogenese (hoofdstuk V en VI) kunnen als volgt worden samengevat. De vroege plaat-vormige cerebellum-aanleg is een pseudo-gelaagd epitheel van prolifererende cellen, dat als een matrix kan worden beschouwd. De matrixcellen strekken zich over de gehele breedte van de - nog dunne - wand uit. Een gedeelte van de wand, nl. een smalle zône aan weerszijden van het mediane vlak (matrixzône M), zal deze kenmerken gedurende de gehele ontwikkeling tot in de volwassen toestand blijven behouden. De overige delen van de wand gaan een perifeer van de matrixlaag gelegen mantellaag vormen. De matrix neemt op deze plaatsen dan niet meer de gehele breedte van de wand in en wordt derhalve ventriculaire matrix genoemd. Gedurende de verdere ontwikkeling gedragen niet alle delen van deze matrix zich op gelijke wijze. Het grootste deel van de ventriculaire matrix zal geheel gebruikt worden voor de vorming van een mantellaag, zodat de laag van prolifererende cellen aan het ventrikeloppervlak geleidelijk dunner wordt en tenslotte verdwijnt. Daarentegen blijven de matrix die de recessus laterales begrenst (matrixzône L) en het ermee verbonden velum medullare posterius (matrixzône P), die beide eveneens een mantellaag vormen, als laag van prolifererende cellen bestaan. De mantelcellen worden door de matrices in radiaire richting afgegeven, d.w.z. evenwijdig aan de orientatie van de matrixcellen. De cellen van de matrixzônes L en P zijn in hoofdzaak caudorostraal georienteerd, met als gevolg dat mantelcellen hier naar rostraal worden afgegeven.

We kunnen de hierboven geschetste ontwikkeling, nl. de vorming van een mantellaag perifeer van de matrixlaag en het verdwijnen van de ventriculaire matrix in bepaalde gebieden, als de eerste fase van de histogenese bestempelen (fig. 14).

In de tweede fase, die reeds begint voor de eerste fase geheel beëindigd

is, migreren nieuw geproduceerde matrixcellen, afkomstig van de matrixzônes M, L en P weg uit hun oorsprongsgebieden en vormen zo een secundaire matrix, die ook daar waar de ventriculaire matrix reeds is uigeput voor toename van cellen zal zorgdragen (fig. 15). De richting waarin de secundaire matrixcellen migreren is verschillend, afhankelijk van het oorsprongsgebied. Matrixzône M zendt de cellen naar lateraal in een tangentieel vlak; een groot deel migreert dicht onder het meningeale oppervlak. De secundaire matrixcellen afkomstig van de matrixzônes L en P migreren in hoofdzaak rostraalwaarts en wel ôf eerst in tangentiële richting, ôf direct radiair, dus in dezelfde richting als waarin tijdens de eerste fase van de ontwikkeling de mantelcellen werden afgegeven. Een geheel extern (d.w.z. aan de meningeale zijde) gelegen laag, zoals beschreven voor vogels en zoogdieren, wordt door de secundaire matrixcellen dus niet gevormd.

Migratie van cellen in radiaire en waarschijnlijk ook tangentiële richting vindt in eerste instantie plaats op geleide van glia uitlopers. Naarmate het cerebellum rijpt kunnen ook andere structuren een geleidende functie vervullen. Een opvallend kenmerk van migrerende cellen is de aanwezigheid van een dicht filamenteus netwerk in hun cytoplasma. Daar deze cellen bovendien zeer veel vrije ribosomen bevatten, zijn ze in electronenmicroscopische en semidunne coupes donker gekleurd (fig. 6, 12, 47, 48).

Het electronenmicroscopisch onderzoek van de histogenese bracht aan het licht dat groei van de meeste, zo niet alle celtypen plaatsvindt door middel van "growth cones" en filopodia. Growth cones bevatten veel vesiculair en tubulair endoplasmatisch reticulum. In de filopodia wordt een filamenteus netwerk aangetroffen (fig. 43).

Na deze algemene opmerkingen zullen de verdere resultaten van het histogenetisch onderzoek puntsgewijs samengevat worden.

1. Zoals vergelijking met de litteratuur aantoont, komt de ultrastructuur van

de langwerpige matrixcellen sterk overeen met die van matrixcellen in andere diergroepen en andere hersendelen (fig. 41, 46). Opvallend zijn de vele vrije ribosomen en de geringe hoeveelheid ergastoplasma. Deling van matrixcellen vindt voornamelijk plaats aan het ventriculaire oppervlak; niet-ventriculaire mitosen zijn evenwel ook aanwezig.

2. De eerste mantelcellen (die ronder van vorm zijn dan matrixcellen, fig. 46) zijn waarschijnlijk alle neuroblasten; structuren met de karakteristieken van axonen worden gevonden zodra een mantellaag wordt gevormd. De eerst glia-differentiatie is in een wat later stadium te zien in uitlopers die contact hebben behouden met het meningeale oppervlak. Glioblasten bezitten en ontwikkelen meer plaatsen van gespecialiseerd contact (niet-synaptische "junctions") dan neuroblasten. Het is mogelijk dat de aanwezigheid van dergelijke contacten of, in het andere geval, hun afwezigheid, invloed heeft op de differentiatie van de betrokken cellen.

Het lijkt een algemeen principe te zijn, dat de zich ontwikkelende cellen in het cerebellum op grond van de structuur van hun uitlopers eerder geidentificeerd kunnen worden dan op grond van de structuur van hun somata. Dit geldt ook voor neuroblasten en glioblasten. Uitlopers van glioblasten zijn te onderscheiden van axonen en dendrieten door het bijna geheel ontbreken van microtubuli in de eerstgenoemde structuren (fig. 46). De perikarya van neuroblasten en glioblasten (mantelcellen) bezitten gedurende lange tijd alle dezelfde ultrastructuur. Vergeleken met matrixcellen is er geen differentiatie te zien, maar wel kan, door de afronding van de mantelcellen, de positie van de cytoplasmatische organellen ten opzichte van de kern verschoven zijn.

3. De neuroblasten die in de eerste fase van de cerebellaire ontwikkeling gevormd zijn, differentiëren tot grote neuronen: Purkinje, eurydendroide en Golgi cellen. Differentiatie van de somata is gekenmerkt door toename van organellen, in het bijzonder ergastoplasma, en gaat gepaard met groei zowel van

de hele cel als van de kern (fig. 46). Uitgroei van dendrieten, die wat meer organellen bezitten dan axonen, begint juist voordat deze differentiatie zichtbaar wordt. De dendrietbomen van volwassen eurydendroide cellen strekken zich over een groot deel van de moleculaire laag uit, vandaar de naam. De eurydendroide cellen nemen een sleutelpositie in de neuronale circuits van het cerebellum in (zie punt 8).

4. De glioblasten, die waarschijnlijk langer contact houden met het ventriculaire en/of meningeale oppervlak dan neuroblasten, differentiëren tot Golgi epitheelcellen, ependymcellen en verschillende typen astrocyten, die op grond van hun vorm te onderscheiden zijn. Overgangsvormen tussen genoemde gliatypen komen voor. Een gemeenschappelijk kenmerk is het bezit van meer of minder brede uitlopers, waarvan de vertakkingen onderling loodrechte hoeken maken. Ook de ultrastructuur van al deze typen is gelijk. Differentiatie wordt gekenmerkt door toename van organellen, maar in minder sterke mate dan in neuronen (fig. 46). Ook hier gaat differentiatie gepaard met groei.

De oorsprong van de overige glia-typen: oligodendrocyten, astrocyten met dunne uitlopers en microgliacyten, uit de ventriculaire matrixlaag kon niet worden vastgesteld.

5. De secundaire matrixcellen worden tot neuroblasten, die differentiëren tot de kleine neuronen van het cerebellum: granulaire cellen (veruit het grootste aantal) en stercellen. In het algemeen is het zo dat de somata van toekomstige granulaire cellen door de differentiërende ganglionaire laag (waarin Purkinje en eurydendroide cellen gelegen zijn) heen migreren, en een eigen laag of celmassa, de granulaire laag, vormen (fig. 15). De moleculaire laag, die opgebouwd wordt tussen de ganglionaire laag en de secundaire matrix, bestaat uit uitlopers van de cellen gelegen in de ganglionaire en de granulaire laag, en bevat bovendien de stercellen.

Er zijn redenen om aan te nemen, dat gedurende de ontwikkeling interactie

optreedt tussen de Purkinje dendrieten en hun afferenten, de axonen van de granulaire cellen. De rijping van granulaire en stercellen wordt weer gekenmerkt door toename van organellen, maar in geringere mate dan in grote neuronen.

Of ook glia uit de secundaïre matrix ontstaat is onduidelijk.

6. De lobus vestibulolateralis is een derivaat van de matrixzônes L en P (fig. 14, 15). Aangezien de cellen in min of meer rostrale richting worden afgegeven, ontstaan vóór de recessus laterales verdikkingen van de zijwand van de hersenen. De ophopingen van granulaire cellen, die in de tweede fase van de histogenese door matrixzône L worden geproduceerd, vormen als eminentiae granulares de rostrolaterale delen van de lobus vestibulolateralis.

7. De nucleus cerebelli, beschreven door Pearson ('36), is afkomstig van de ventriculaire matrix die de angulus lateralis van de ventrikel in het isthmusgebied omgeeft (fig. 16).

8. In het corpus cerebelli werd de ontwikkeling van de neuronale verbindingen onderzocht. De eerste synapsen, die gevonden worden op de dag dat de embryonen uit het ei komen, zijn axodendritische contacten tussen parallelvezels en de schacht van Purkinje dendrieten. Deze synapsen zijn echter van voorbijgaande aard, daar in latere stadia parallelvezels slechts synapsen vormen met de "spines" van deze dendrieten. De eerste axosomatische synapsen verschijnen wat later dan de eerste axodendritische en zijn contacten tussen Purkinje axonen en de cellen van de ganglionaire laag. Nog later worden klimvezel- en mosvezelsynapsen gevormd. De axonen van stercellen maken als laatste hun contacten met de dendrieten en somata van Purkinje cellen.

De mosvezels vormen het voornaamste input-systeem; de door deze vezels aangevoerde prikkels bereiken via de granulaire cellen de moleculaire laag. Klimvezels spelen in het cerebellum van de forel waarschijnlijk een minder belangrijke rol dan mosvezels. De zich in de moleculaire laag uitbreidende

dendrieten van Purkinje, eurydendroide, Golgi en stercellen, synapteren met parallelvezels. De axonen van de Purkinje cellen en hun collateralen verlaten het cerebellum niet, doch blijven binnen de ganglionaire laag, terwijl een deel van hun collateralen zich in de moleculaire laag uitbreidt. In de ganglionaire laag synapteren de Purkinje axonen voornamelijk met de somata en de hoofddendrieten van de eurydendroide cellen. De axonen van de laatstgenoemde elementen vormen waarschijnlijk het belangrijkste output-systeem van het corpus cerebelli. Een diagram van de neuronale verbindingen in het corpus cerebelli is weergegeven in figuur 54.

De resultaten van het onderzoek worden besproken in het licht van de huidige opvattingen op het gebied van de neuroembryologie.

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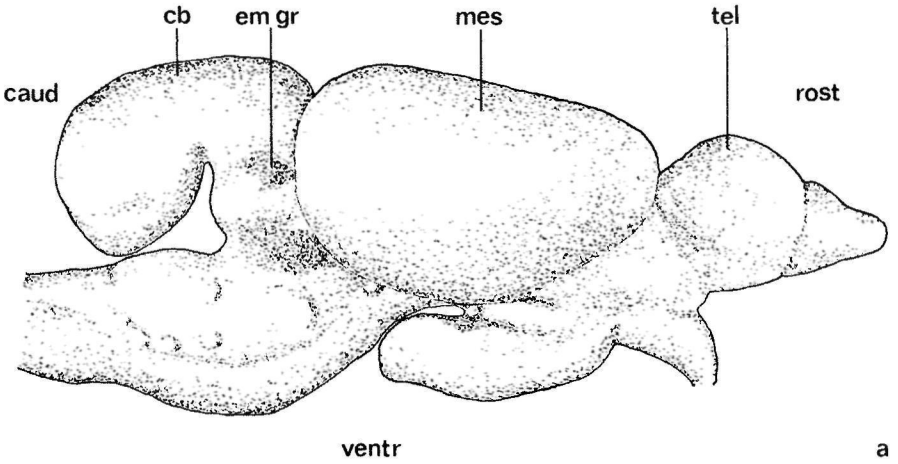
ILLUSTRATIONS

Fig. 1. Lateral (a) and medial (b) view of the brain of a 230 mm trout, based on a three-dimensional reconstruction. X 10. The finely dotted structures in (b) are situated lateral to the median plane. The levels of the transverse sections represented in fig. 2 are indicated in (b).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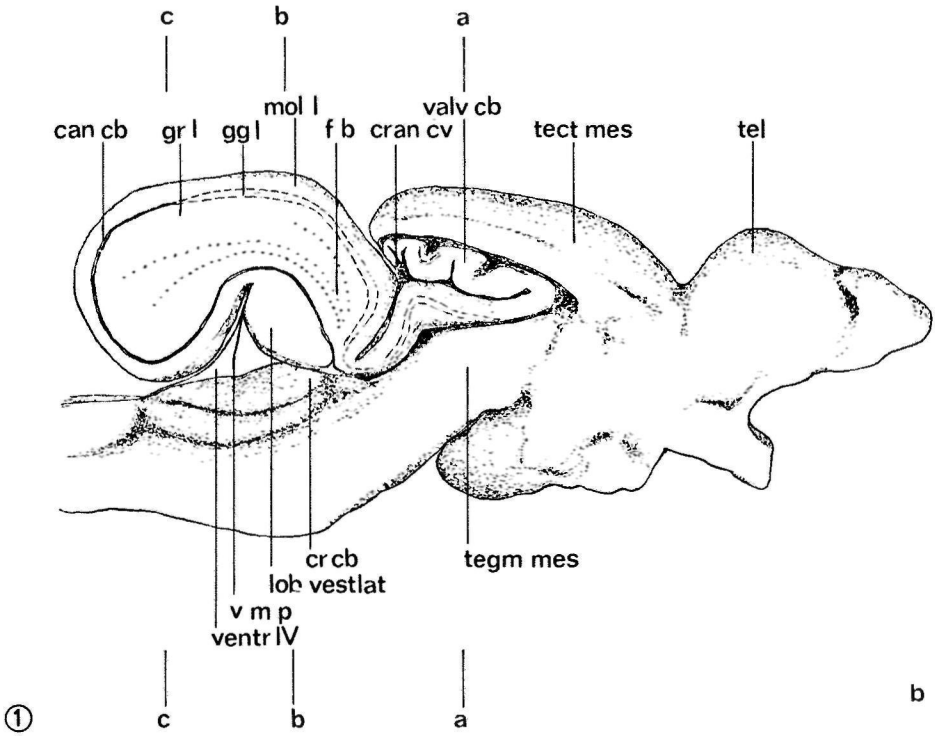
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Fig. 2. Diagrammatic transverse sections through the brain of a 230 mm trout, to show the position of the cerebellar layers. X 12. The levels of the sections are indicated in fig. 1b. (a) valvula cerebelli, (b) corpus cerebelli and lobus vestibulolateralis, (c) corpus cerebelli.

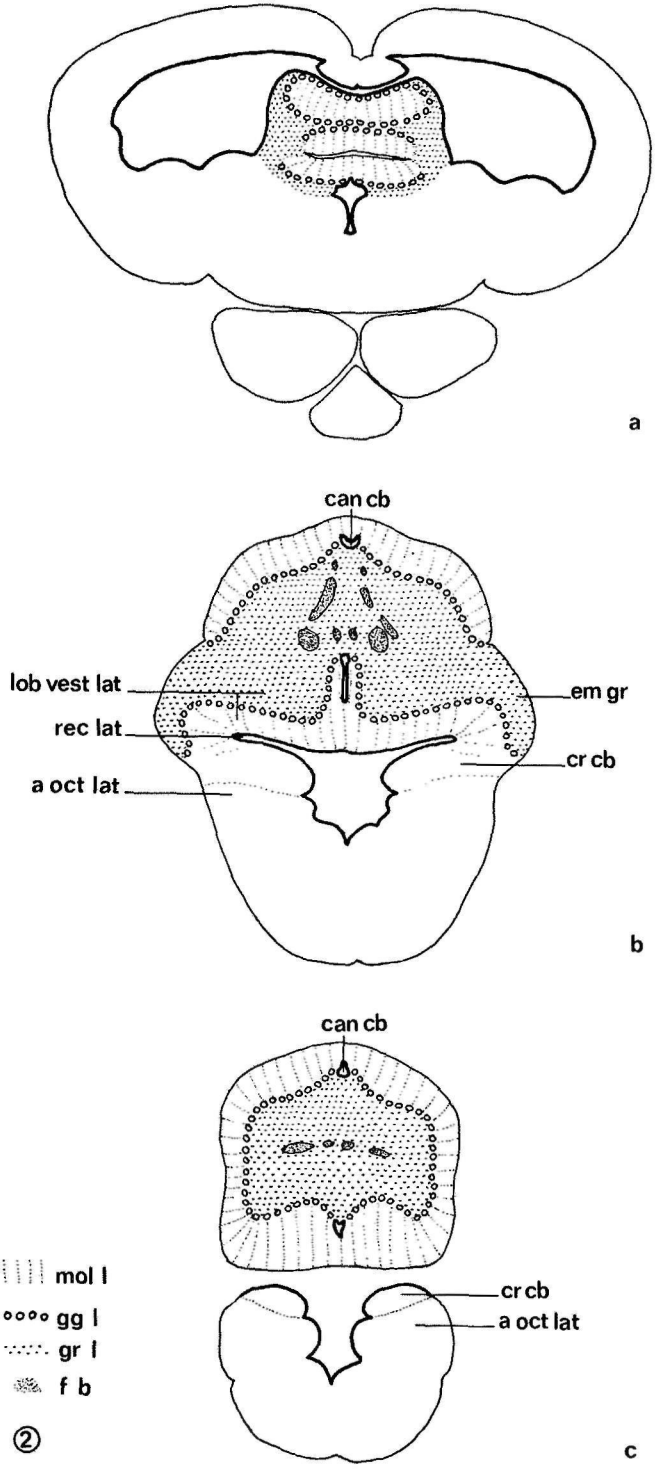
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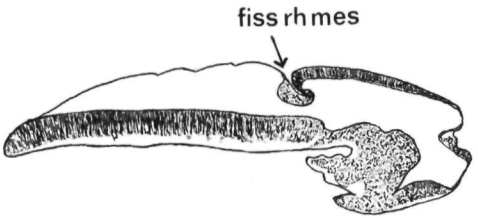
Fig. 3. The development of the cerebellum in median sections. (a) to (f) H.E., (g) Klüver-Barrera. X 30. (a) 7 mm, (b) 10.5 mm, (c) 11.5 mm, (d) 15 mm, (e) 16 mm, (f) 23 mm, (g) 80 mm.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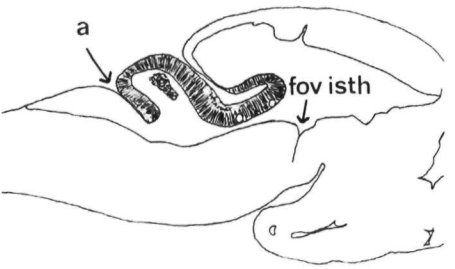
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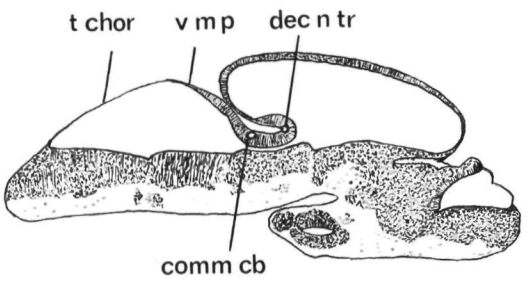
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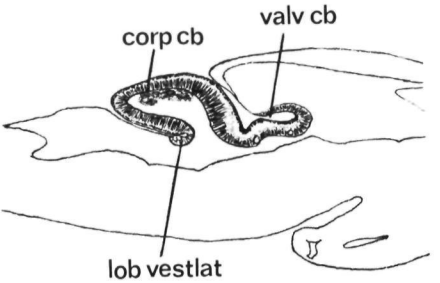
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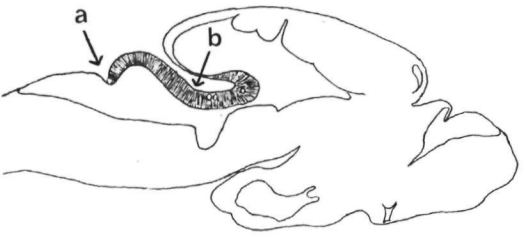
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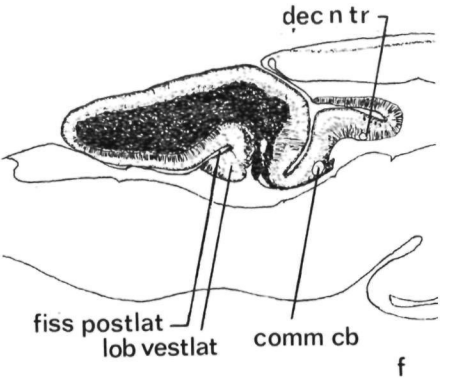
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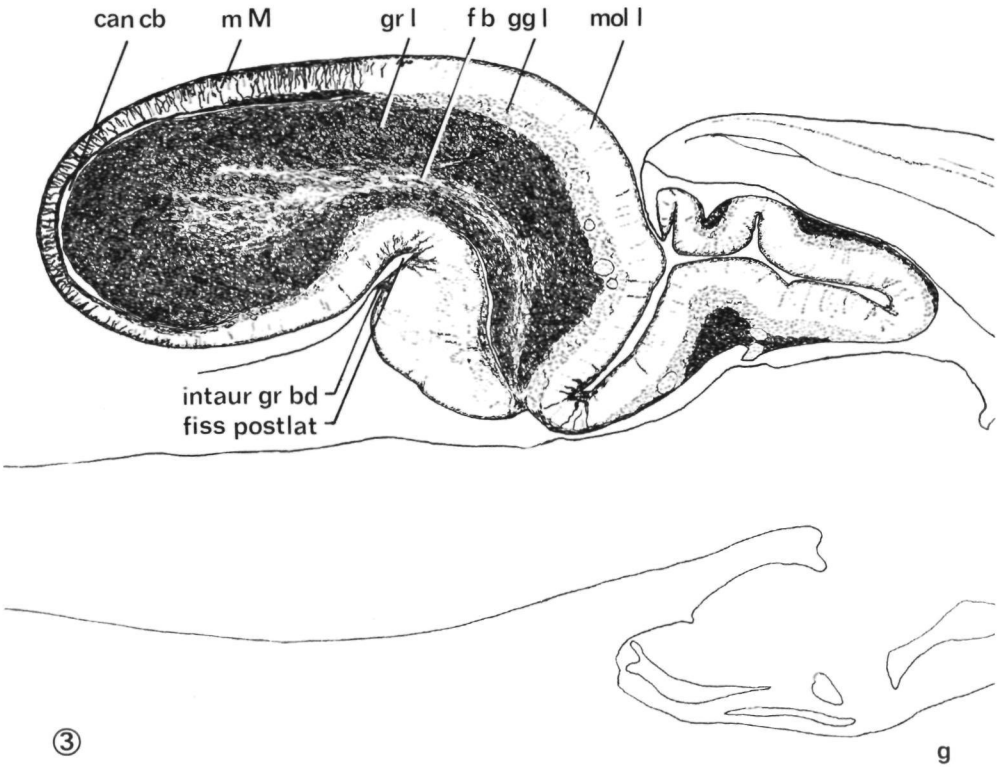
Fig. 4. Dorsolateral view of an embryo of 4.5 mm. X 27.

Fig. 5. Dorsolateral view of an embryo of 7 mm. X 27.

Fig. 6. Semithin (2 μm) sagittal epon section of the cerebellar anlage of a 5.5 mm embryo. Unstained, photographed with phase microscopy. X 575. "Dark cells" are present in the matrix layer and in the mantle layer.


can cb
m M
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③
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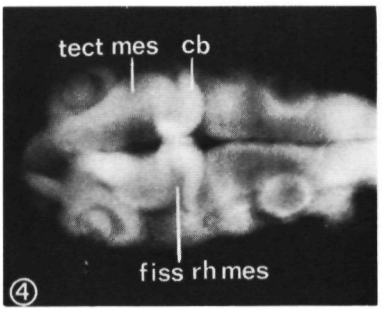
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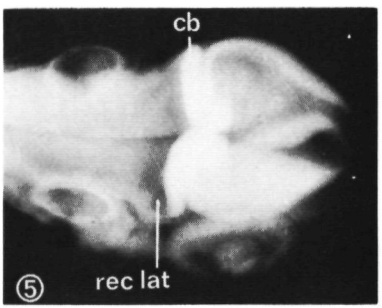
cb
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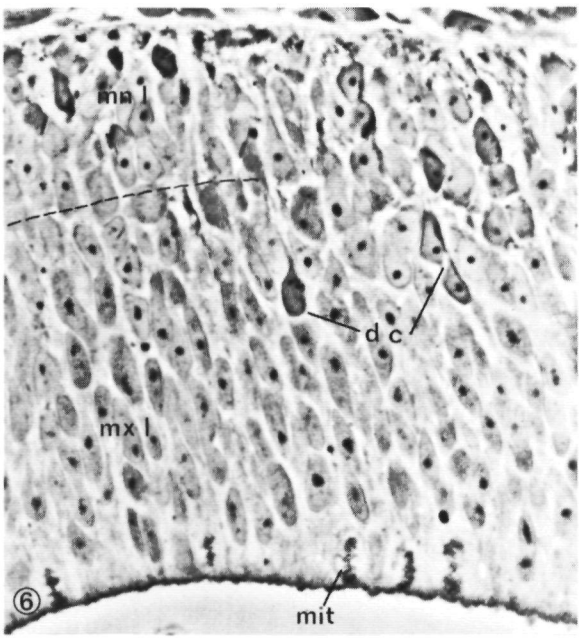
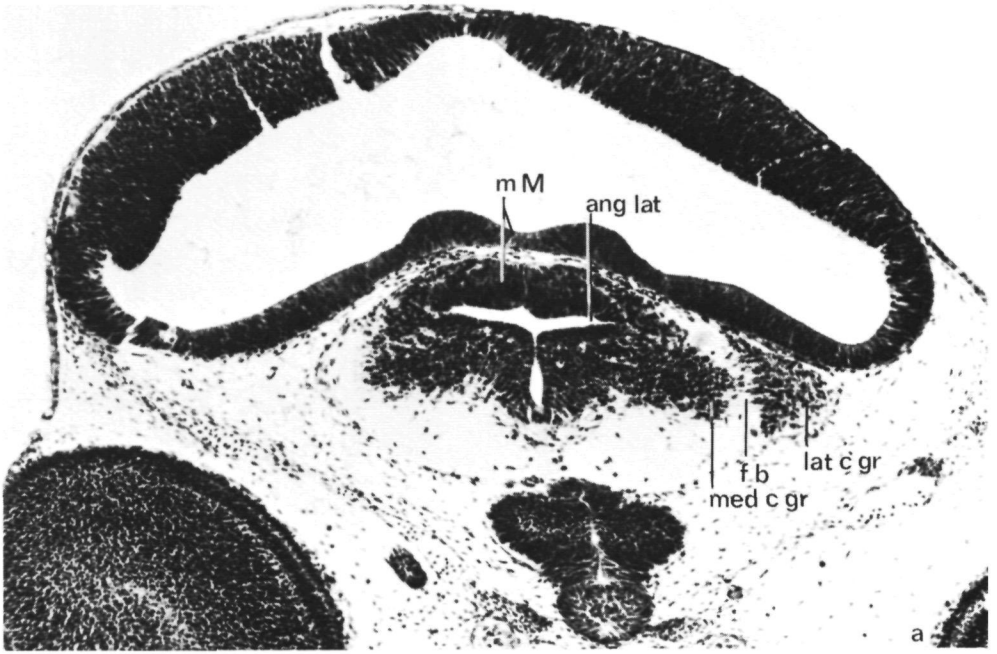

mn l
d c
mx l
⑥
mit

Fig. 7. Transverse sections of a 10.5 mm embryo, at levels from rostral to caudal. H.E. X 95. (a) and (b) primordial valvula cerebelli, (c) and (d) primordial corpus cerebelli.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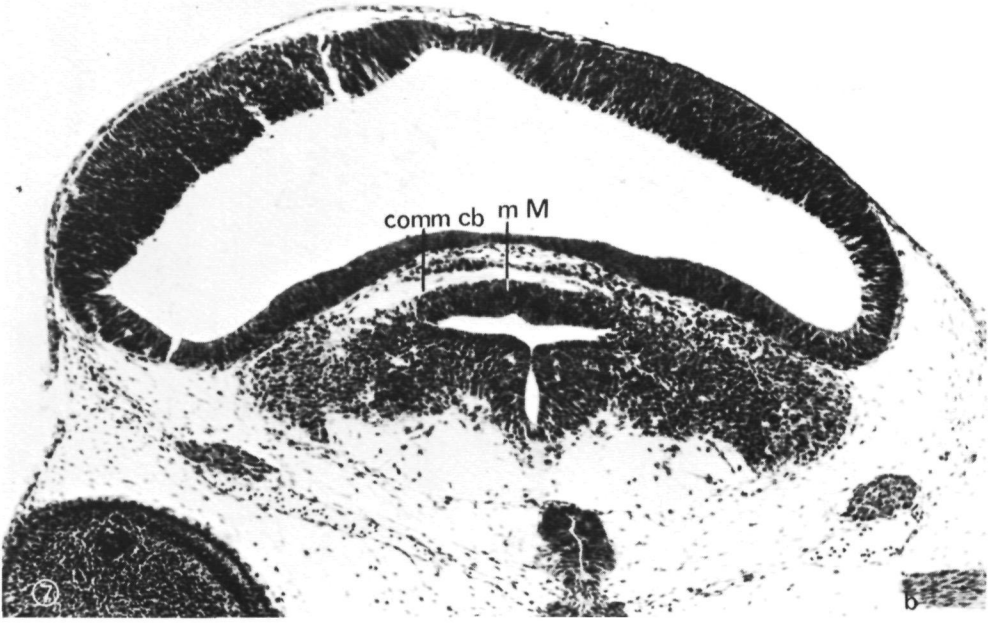
ang lat
f b
lat c gr
med c gr
a


comm cb
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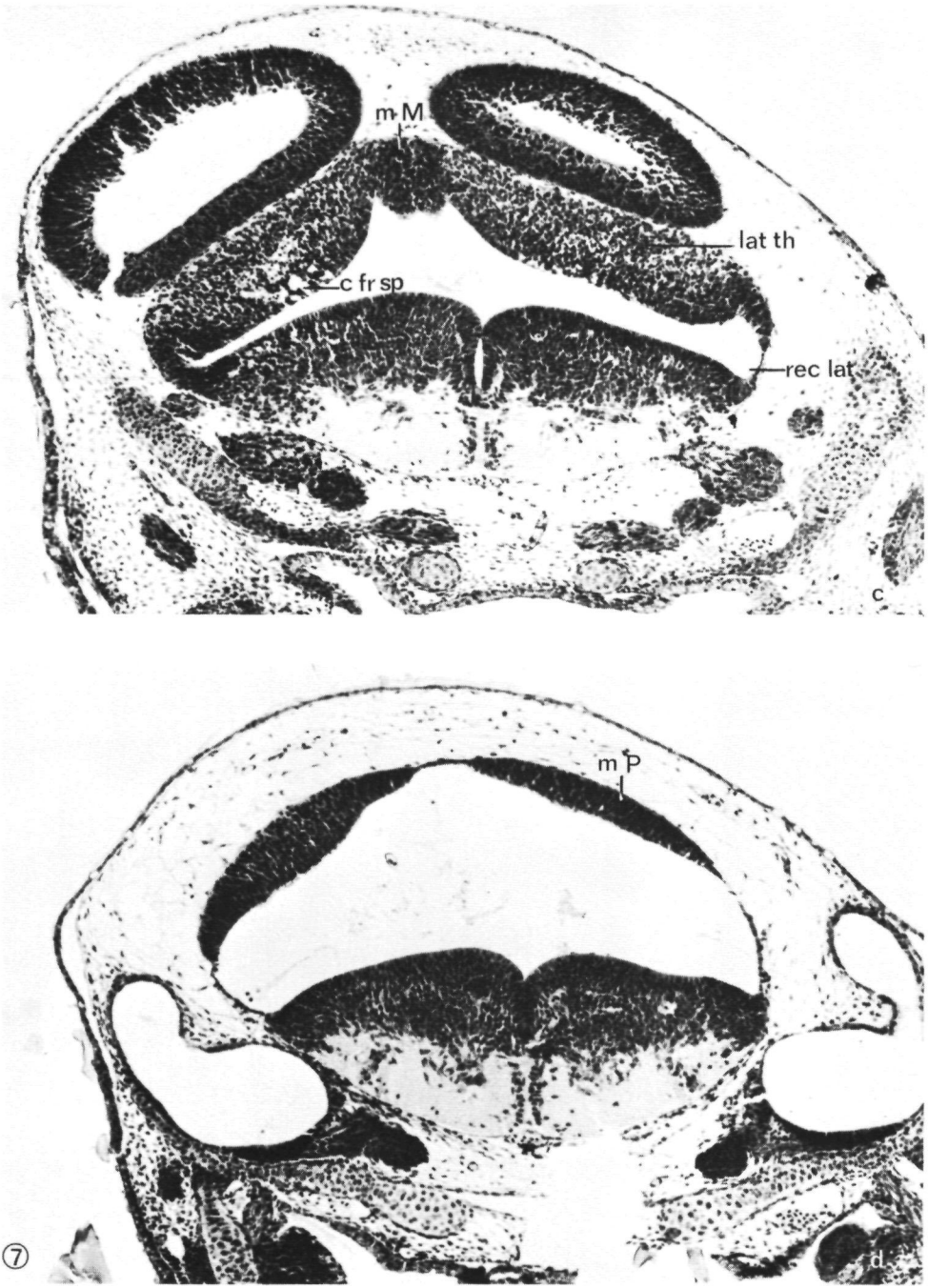
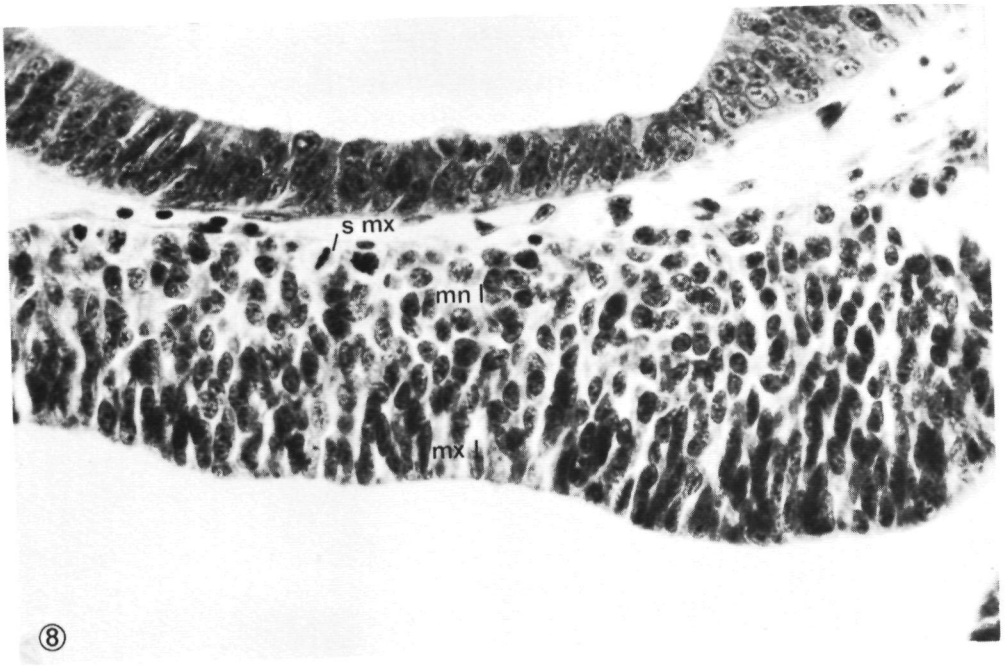


⑦

Fig. 8. Sagittal section through the cerebellar anlage of a 10.5 mm embryo, in the vicinity of matrixzone M. H.E. X 470.

Fig. 9. Sagittal section through the lateral thickening of a 11.5 mm embryo. H.E. X 290.



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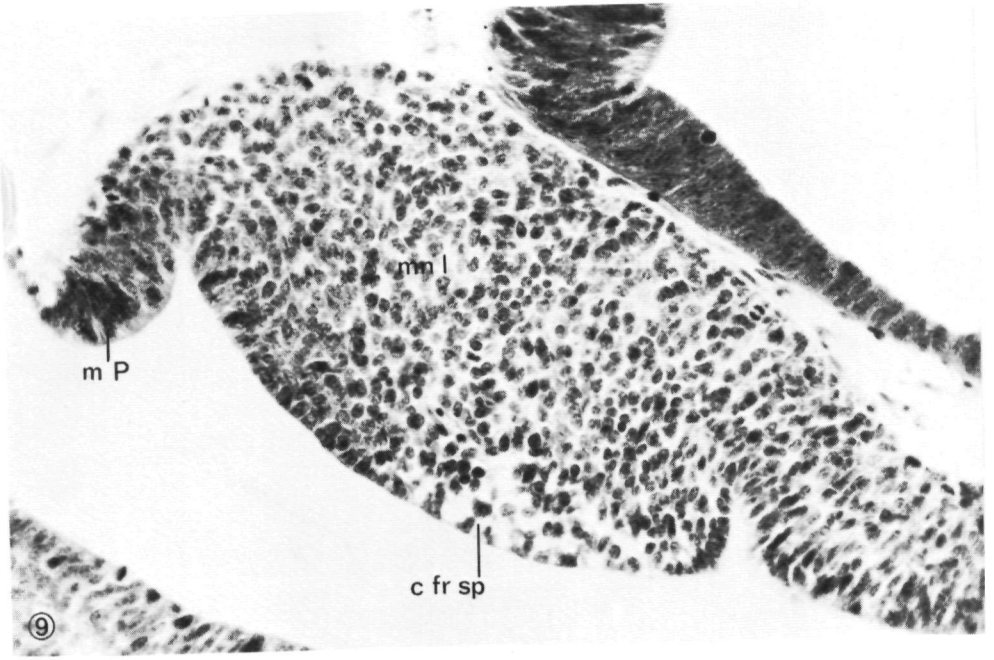

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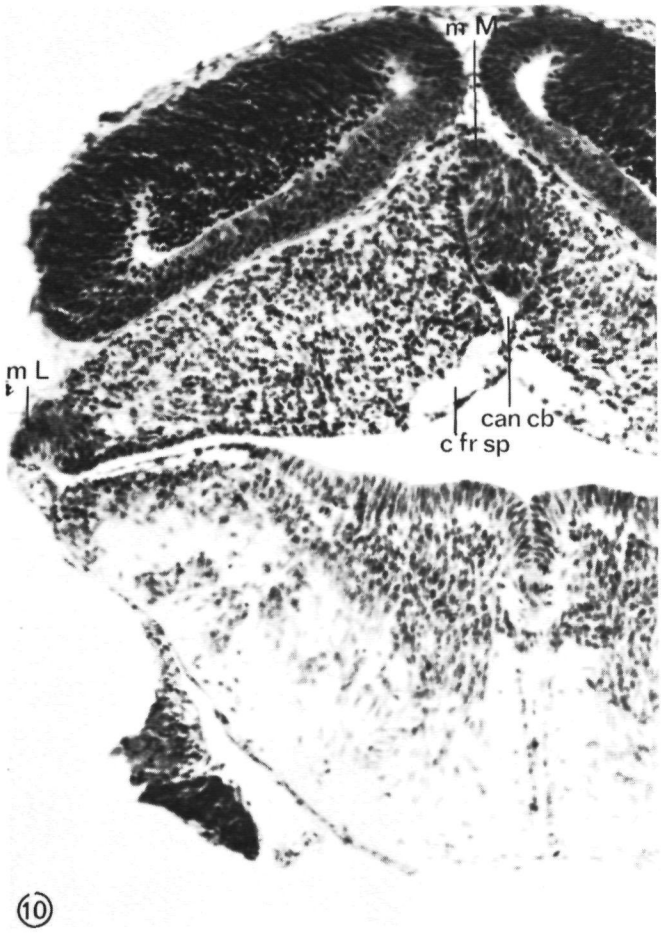
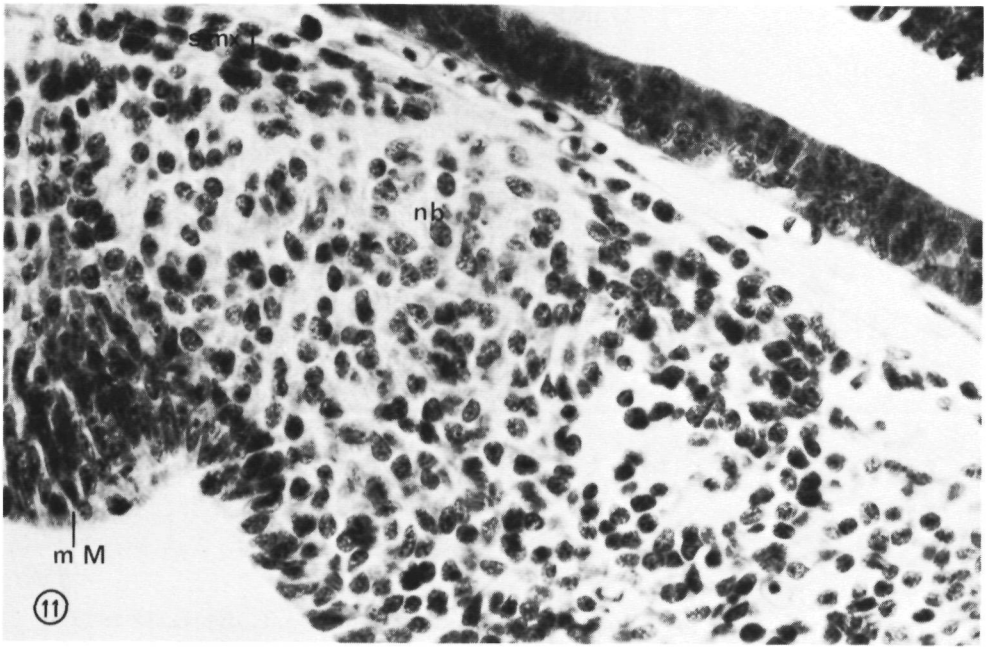
Fig. 10. Transverse section through the corpus cerebelli of a 13 mm trout, at the level of the lateral recesses. H.E. X 145.



⑩

Fig. 11. Transverse section through the rostral part of the corpus cerebelli of a 13 mm trout. H.E. X 470.

Fig. 12. Semithin (2 µm) transverse epon section through the valvula cerebelli of a 13 mm trout. Unstained, photographed with phase microscopy. X 575. "Dark cells" are present in matrixzone M, in the secondary matrix as well as in the mantle layer.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nb
m M
11

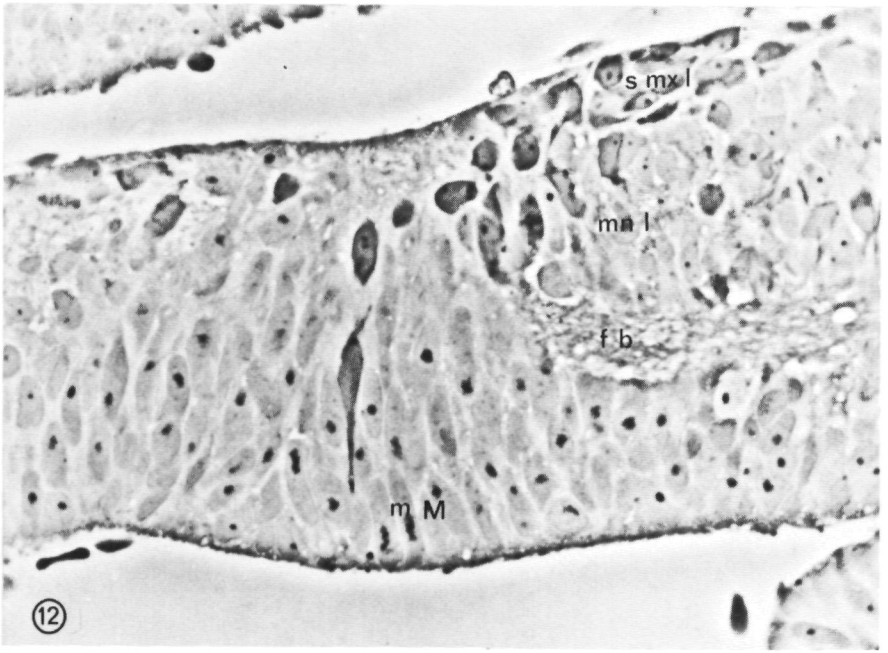
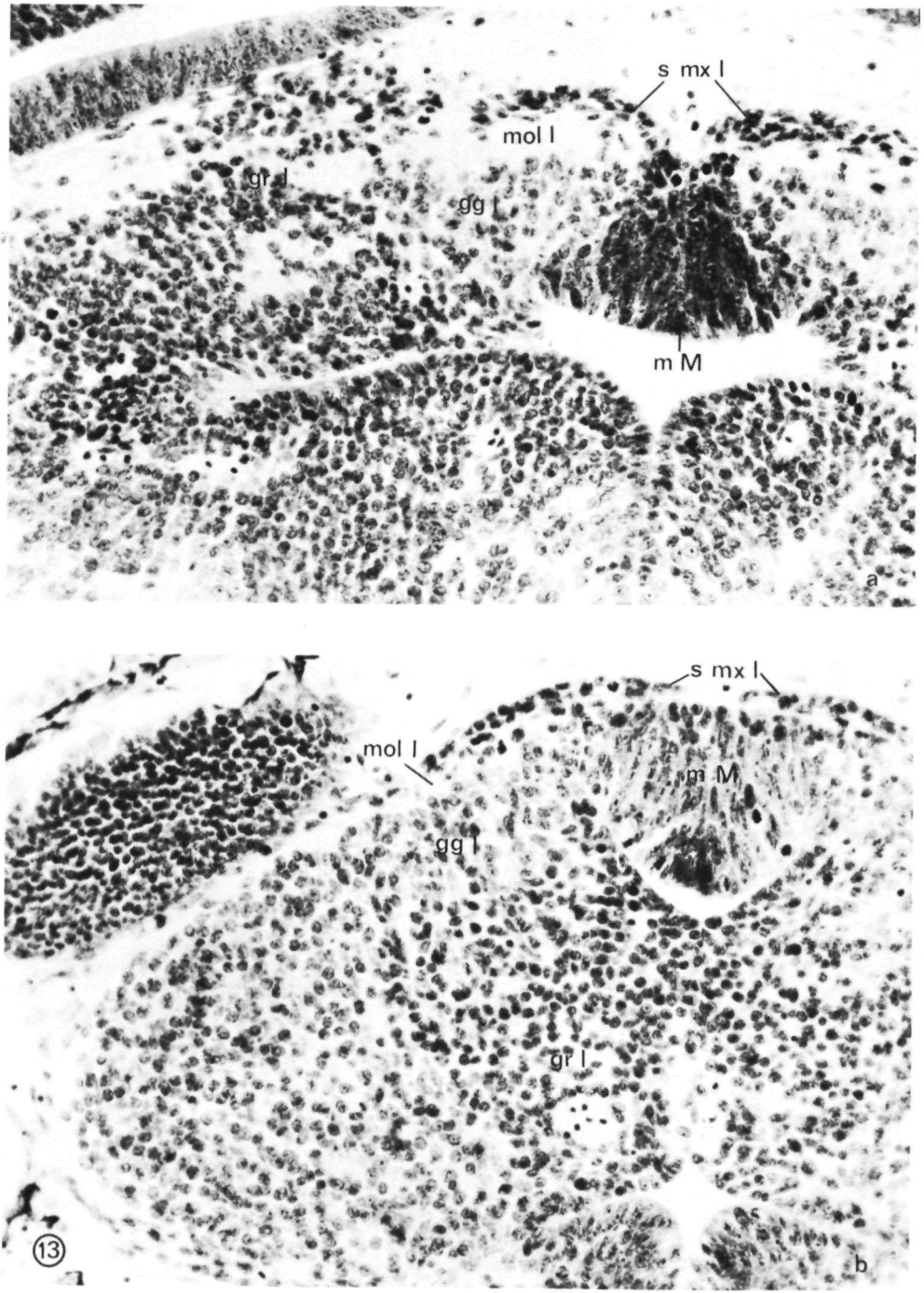

s mx l
mn l
f b
m M
12

Fig. 13. Transverse sections through the valvula (a) and the corpus (b) of a 15.5 mm trout. H.E. X 290.

Fig. 14. Schematic representation of the first phase of histogenesis. Embryo of about 10 mm. The upper left figure shows the cerebellum and the underlying part of the brain stem, the upper right figure the isolated matrix layer of the cerebellum, both of them in a dorsocaudal view. The small central figure represents a median section through the cerebellum. The sections a, b, c, d 1-4 are indicated in the upper and central figures. (a) corpus cerebelli, (b) valvula cerebelli, (c) the region rostral to the lateral recess. The ventricular surface is cut through the angulus lateralis of the ventricle in the isthmus region caudally up to the lateral recess, (d 1-4) the velum medullare posterius (matrixzone P) from medial to lateral. Medially matrixzone P passes into matrixzone M (d1), laterally into matrixzone L (d4). The direction of migration of mantle cells is indicated by short arrows. Further explanation in the text.

Fig. 15. Schematic representation of the second phase of histogenesis. Trout of about 15 mm. The individual figures are directly comparable to those in fig. 14. Comparison of the upper right figure with that of fig. 14 elucidates the curvature of the cerebellum during development, and further, which parts of the matrix become exhausted. The migration paths of secondary matrix cells are indicated by long, slender arrows. Further explanation in the text.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m M
a
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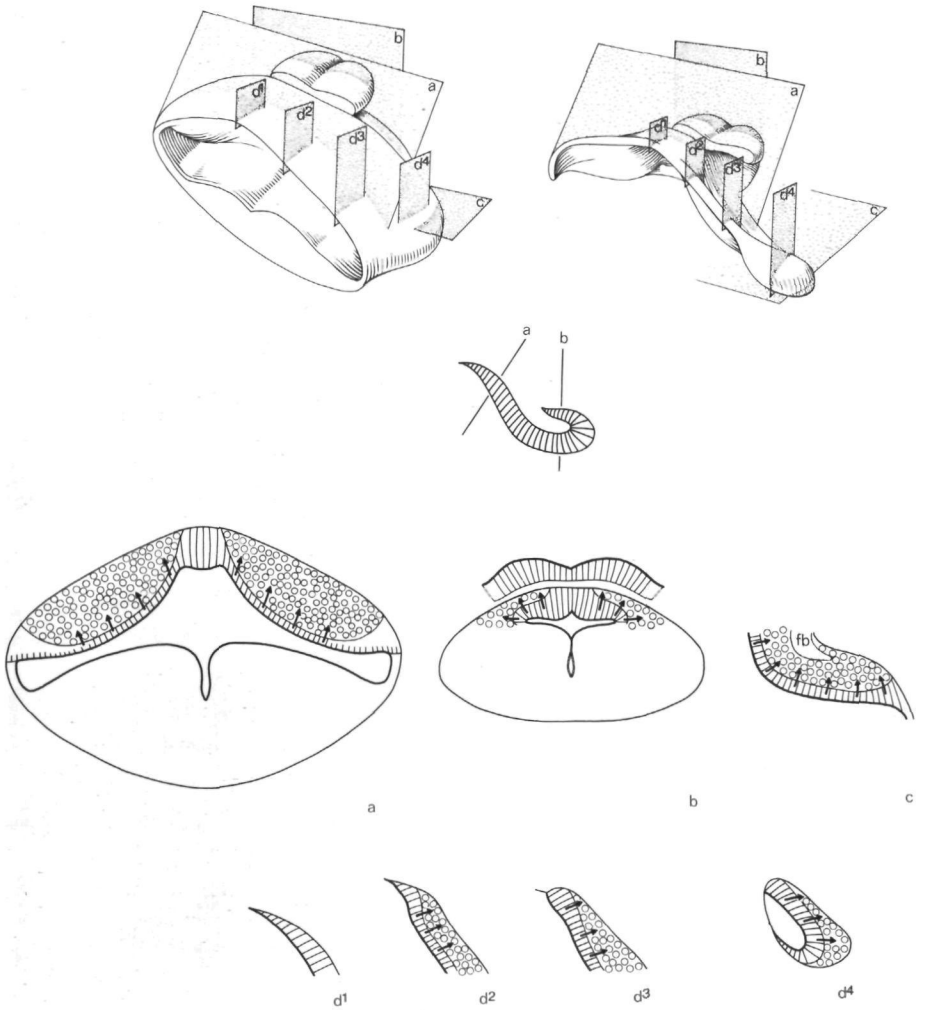
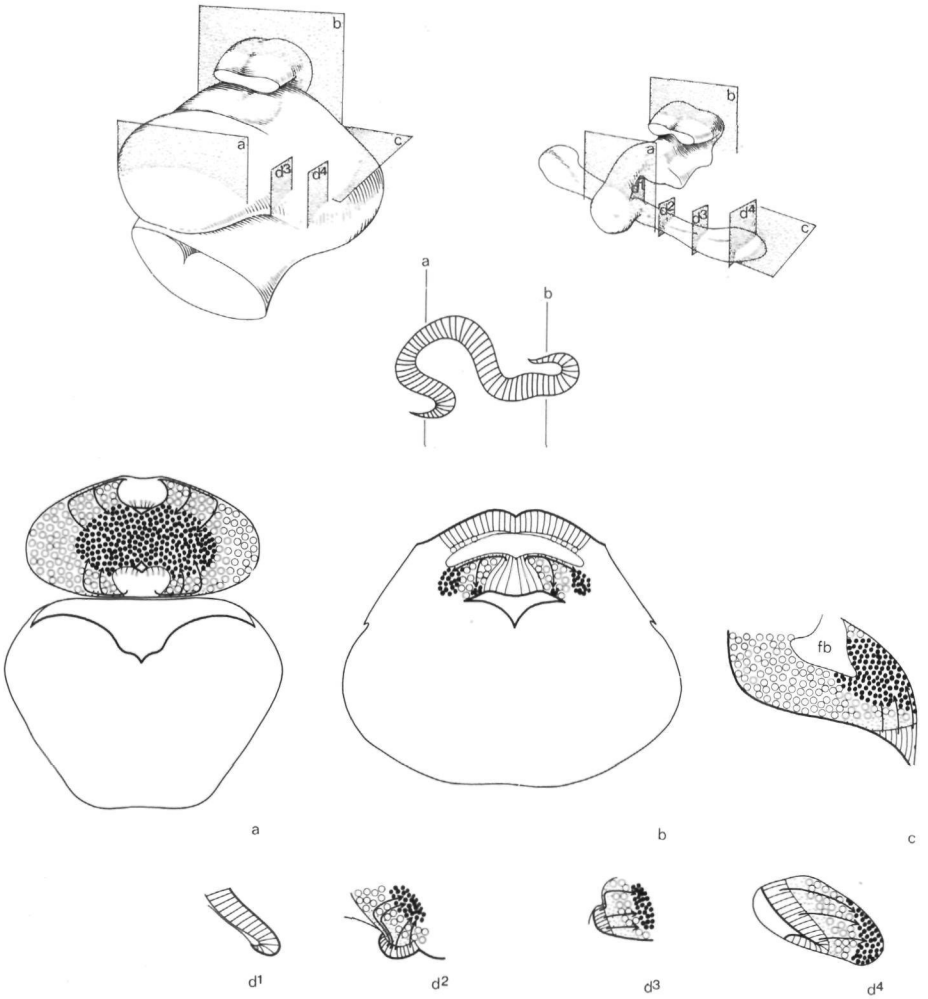
gg l
gr l
13
b



matrix cells, oriented parallel to the plane of sectioning

matrix cells, which are not oriented in the plane of sectioning

mantle layer



matrix cells, oriented parallel to the plane of sectioning

matrix cells, which are not oriented in the plane of sectioning

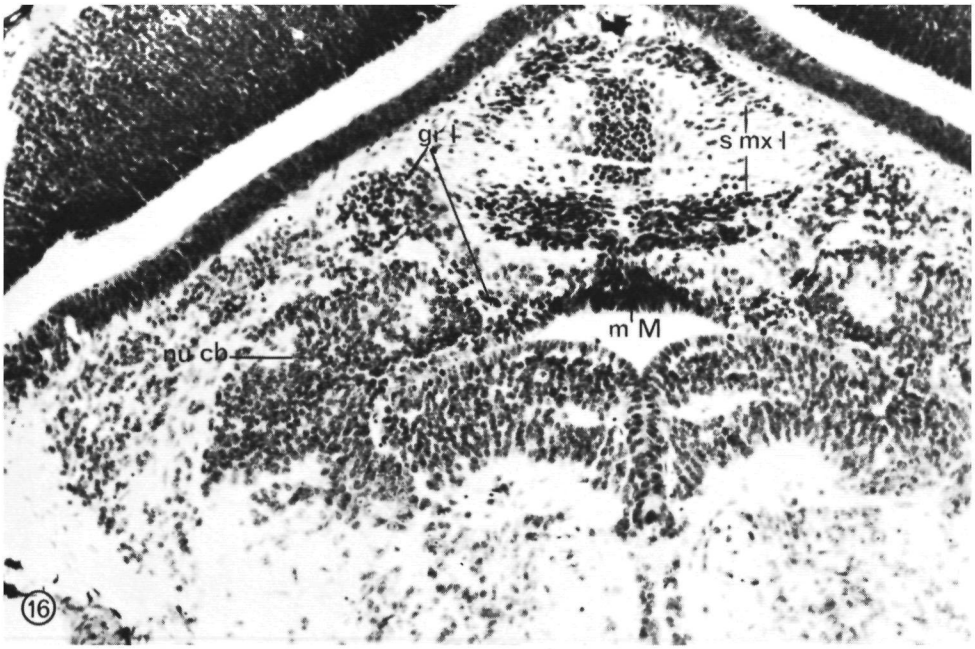
ooo ganglionic layer

..... granular layer

molecular layer

Fig. 16. Transverse section through the caudal part of the valvula cerebelli of a 16 mm trout. H.E. X 145. The anlage of the nucleus cerebelli surrounds the lateral angle of the ventricle.

Fig. 17. Transverse section at the level of the ganglionic layer of the vestibulolateral lobe of a 23 mm trout. H.E. X 145. The continuity of the ganglionic layer of the vestibulolateral lobe with the area octavolateralis is shown.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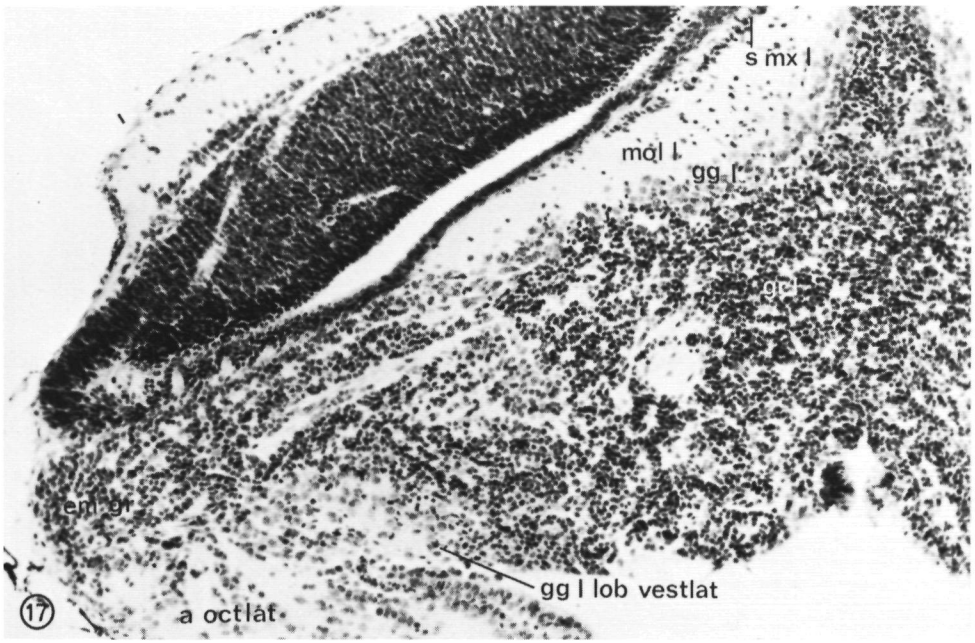
nu cb
16


s mx l
mol l
gg l
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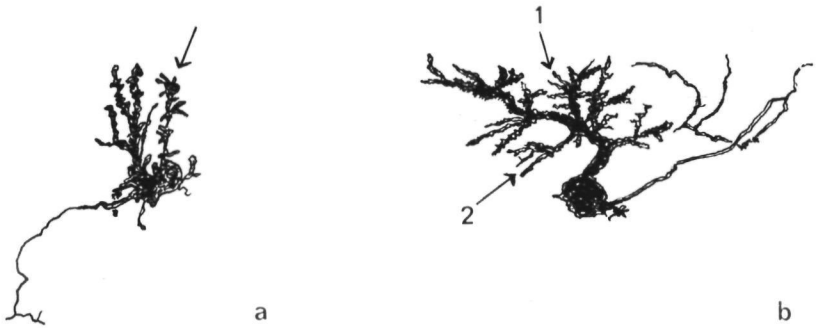
gg l lob vestlat
a octlat
17

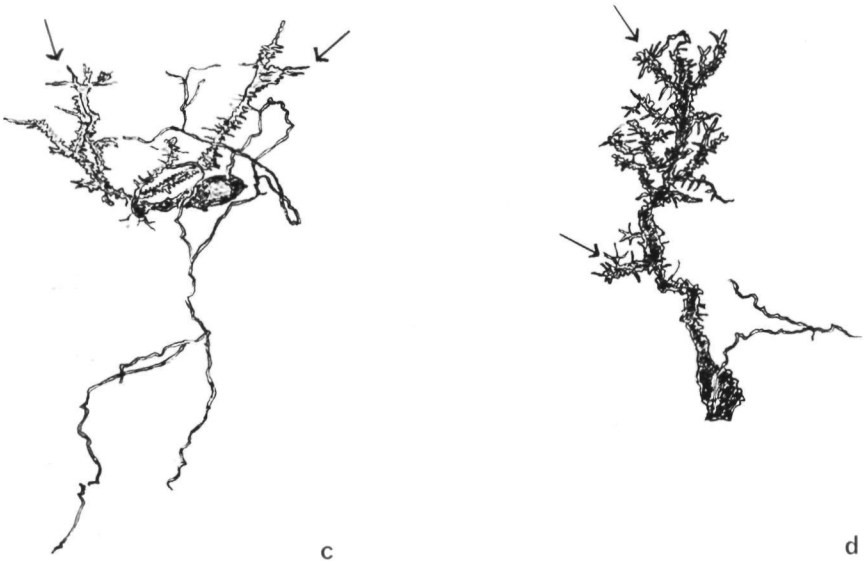
Fig. 18. *(page 189)* Composite drawing of neurons in the mature corpus cerebelli, observed in sagitally sectioned Golgi preparations. X 180.

Fig. 19. The cell bodies and axons of two mature Purkinje cells. Sagittal Golgi preparations. X 120.

Fig. 20. Stages in the development of Purkinje cells, as observed in Golgi preparations. X 480. (a) in the lobus vestibulolateralis; sagittal section of a 20 mm trout, (b) at the transition from corpus to valvula cerebelli; sagittal section of a 20 mm trout, (c) in the corpus cerebelli; transverse section of a 24 mm trout, (d) in the lobus vestibulolateralis; sagittal section of a 24 mm trout. Further explanation in the text.

19

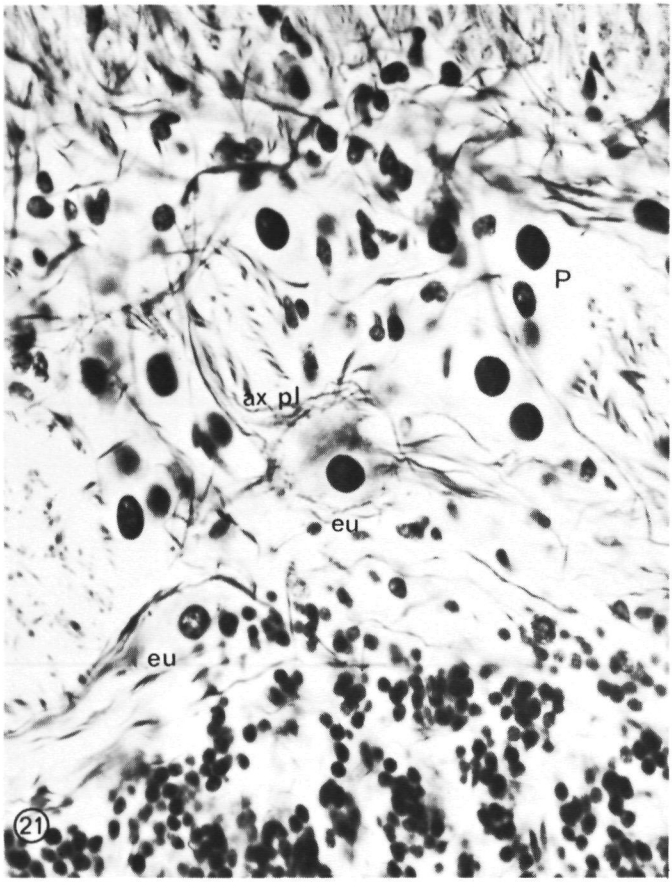




20

Fig. 21. Mature eurydendroid cells in the ganglionic layer of the corpus cerebelli. Sagittal Bodian preparation. X 470.

Fig. 22. Young eurydendroid cells, observed in sagittal Golgi preparations. X 480. (a) in the corpus cerebelli of a 20 mm trout, (b) in the valvula cerebelli of a 24 mm trout.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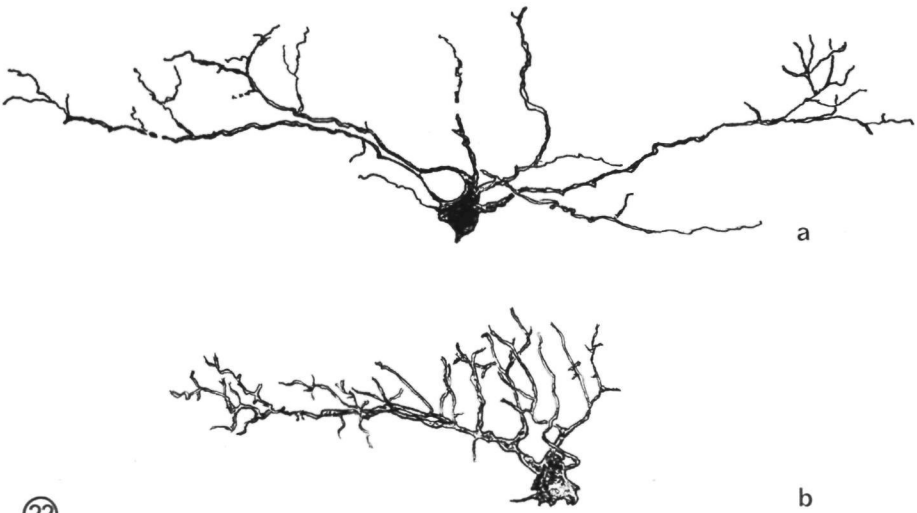
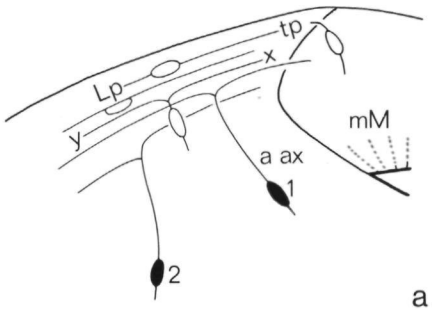
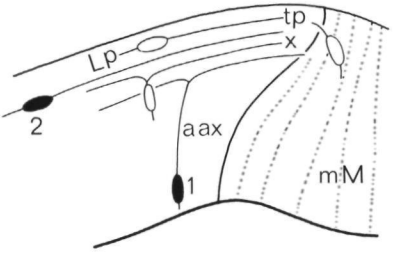

a
b
22

Fig. 23. Schematic representation of the development of granule cells in the trout (a, b, c), compared with the development of granule cells in mammals and birds, according to Cajal (d). (a) corpus cerebelli, (b) valvula cerebelli, (c) the region rostral to the lateral recess. The somata of granule cells that have reached their destination are shown in black. Further explanation in the text.

Fig. 24. Stages in the development of granule cells, as observed in Golgi preparations. X 480. (a) in the valvula cerebelli; transverse section of a 20 mm trout, (b) in the corpus cerebelli; sagittal section of a 24 mm trout.



a



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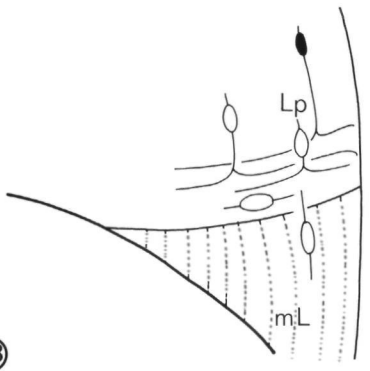

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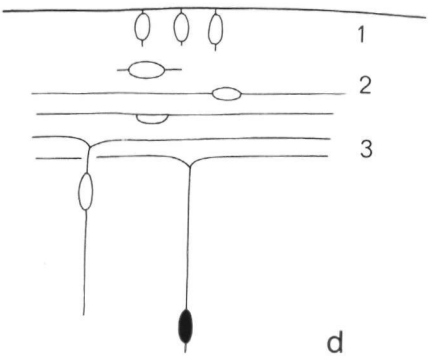
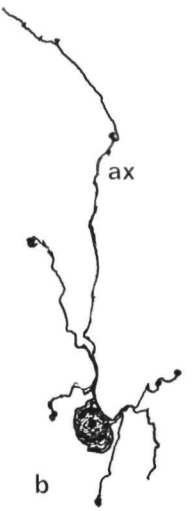


d

23

a

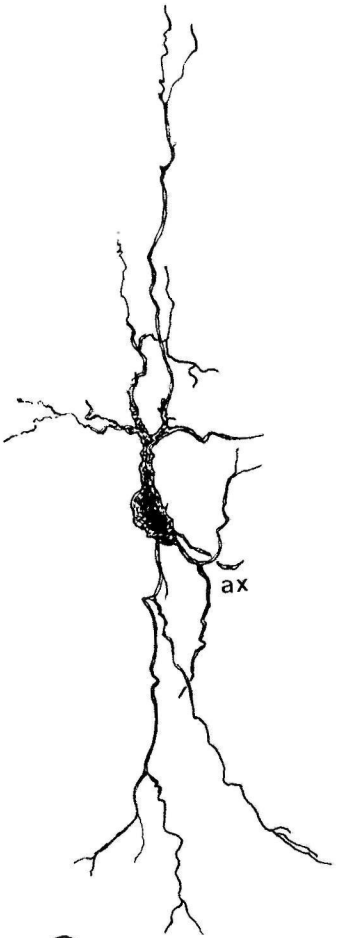


b

24

Fig. 25. Young Golgi cell in the lobus vestibulolateralis of a 20 mm trout. Sagittal Golgi preparation. X 480.

Fig. 26. Stages in the development of stellate cells, as observed in Golgi preparations. X 480. (a) in the corpus cerebelli; transverse section of a 20 mm trout, (b) and (c) in the corpus cerebelli; transverse sections of a 80 mm trout. Arrows indicate T-shaped branching of dendrites.



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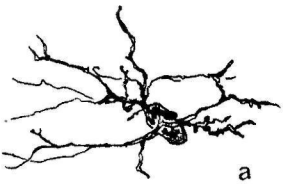
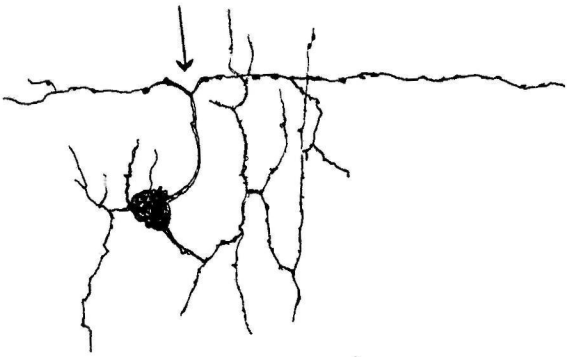
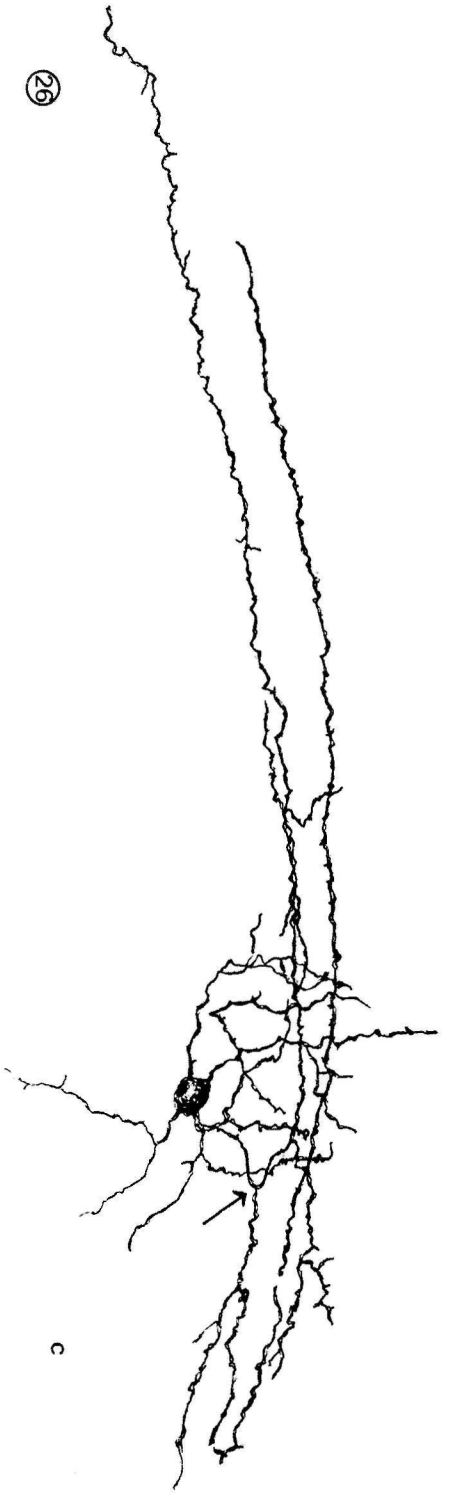




26

Fig. 27. Mature mossy fibre rosette at one focal level. Detail of fig. 18. Sagittal Golgi preparation. X 1200.

Fig. 28. Stages in the development of mossy fibres, as observed in Golgi preparations. X 480. (a) in the corpus cerebelli; sagittal section of a 20 mm trout, (b) in the corpus cerebelli; sagittal section of a 24 mm trout.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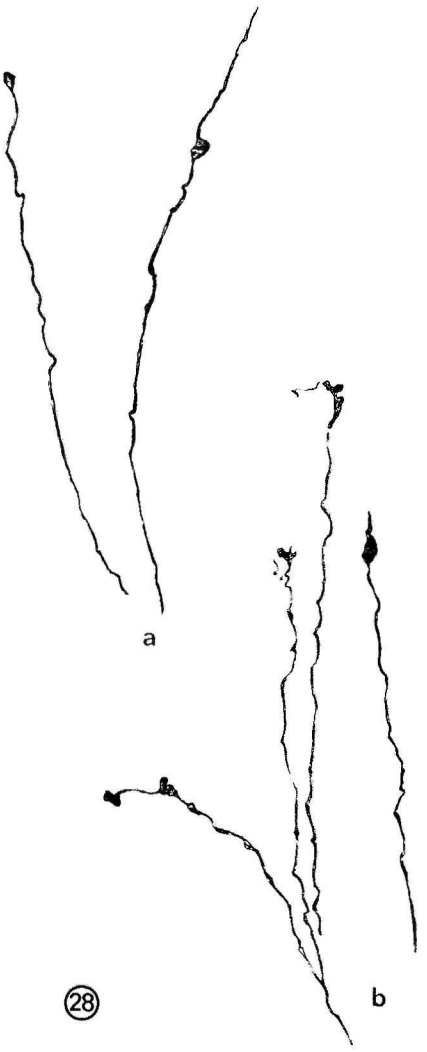

a
28
b

Fig. 29. Young climbing fibres, observed in sagittal Golgi preparations. X 480. (a) in the corpus cerebelli of a 29 mm trout, (b) in the valvula cerebelli of a 32 mm trout.

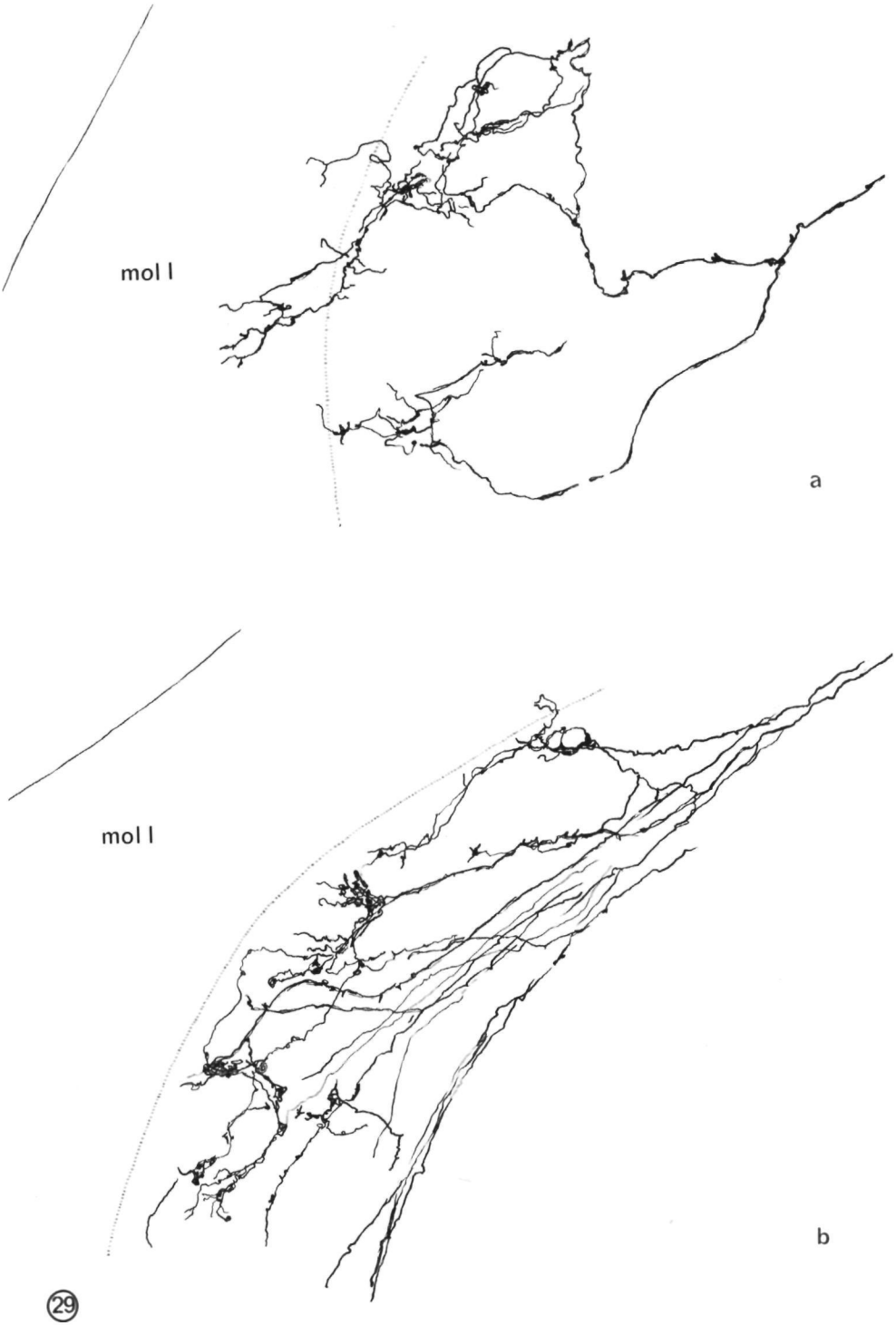
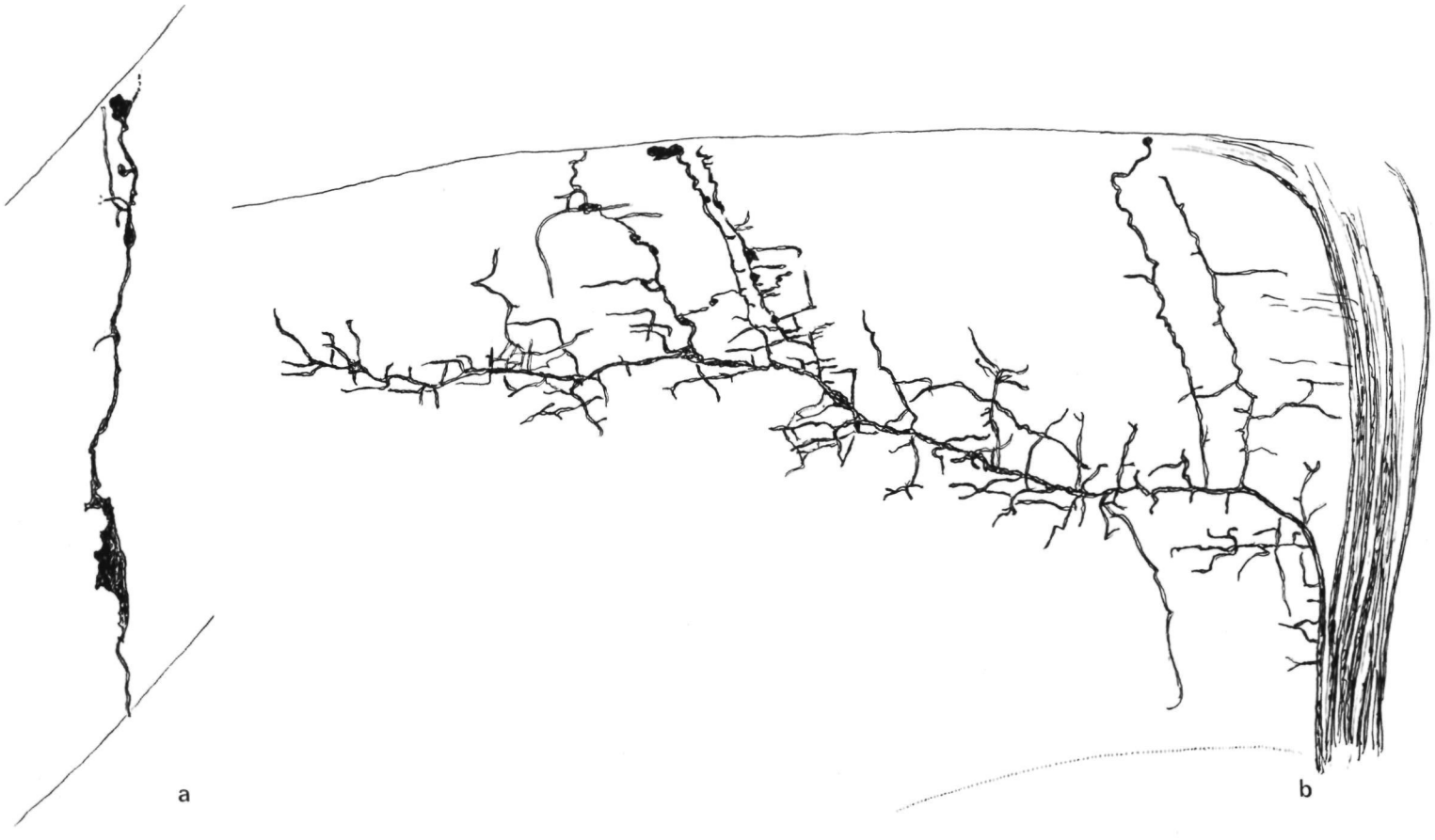

mol l
a
mol l
b

Fig. 30. Mature Golgi epithelial cell in the corpus cerebelli. Sagittal Golgi preparation. X 480.

Fig. 31. Cells of matrixzone M in the mature corpus cerebelli, as observed in Golgi preparations. X 480. (a) presumptive matrix cell; sagittal section, (b) ependymal cell, the soma of which is not visible; transverse section.



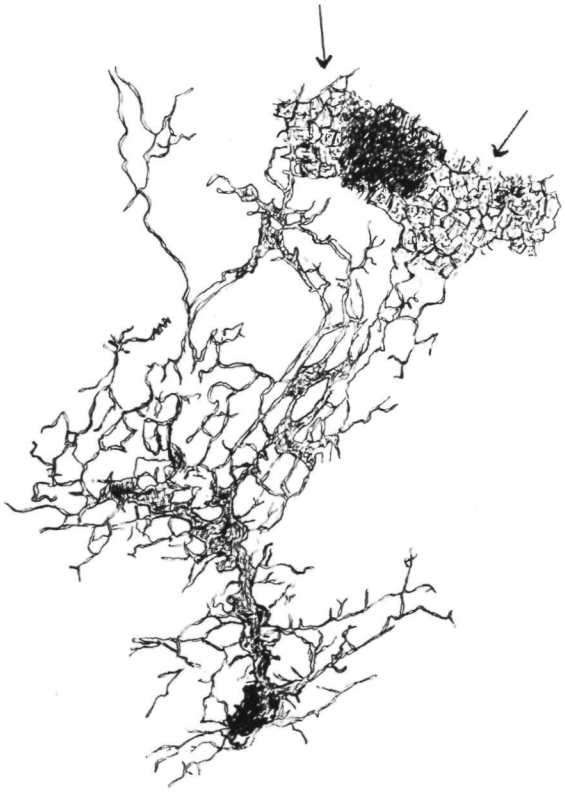
31

Fig. 32. Mature ependymoid astrocyte in the corpus cerebelli. Sagittal Golgi preparation. X 385. Arrows indicate contacts between processes of this cell and blood vessels.

32

Fig. 33. Mature velate protoplasmic astrocyte in the corpus cerebelli. Sagittal Golgi preparation. X 480. Arrows indicate places where septa have been formed. The "lattice" structures formed by the processes of this element can not be delimited from corresponding structures of neighbouring velate astrocytes.

Fig. 34. Mature astrocytoid ependymal cell in the corpus cerebelli. Sagittal Golgi preparation. X 480. The ventricular surface has been indicated.



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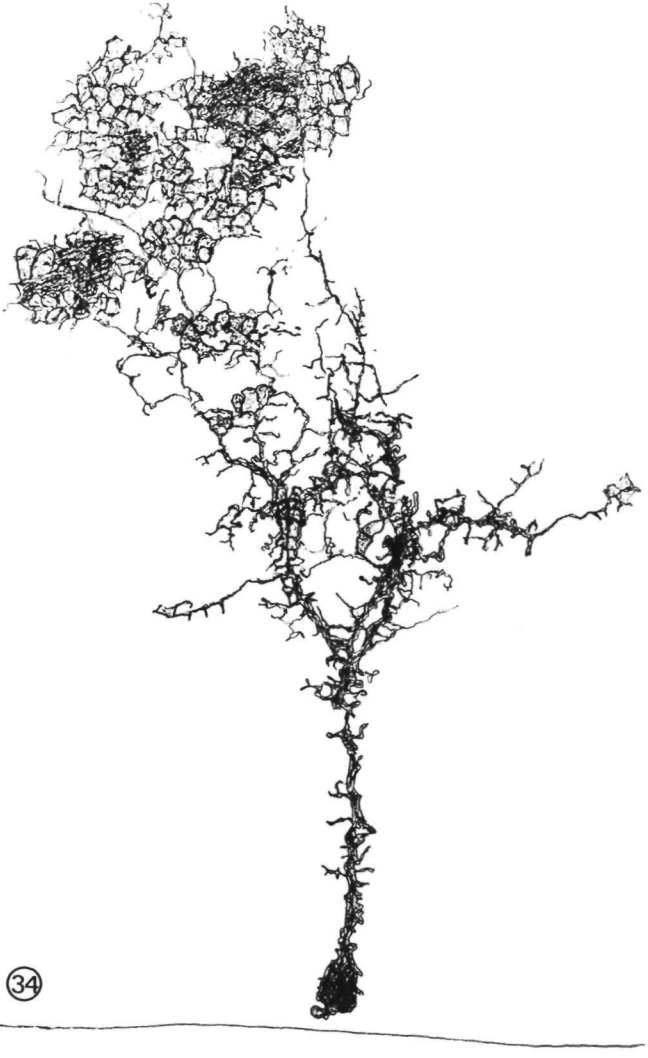
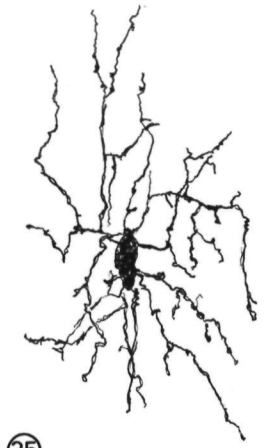


34

Fig. 35. Mature smooth protoplasmic astrocyte in the molecular layer of the corpus cerebelli. Sagittal Golgi preparation. X 480.

Fig. 36. Mature oligodendrocytes in the corpus cerebelli. Sagittal Golgi preparations. (a) between fibre bundles of the granular layer; X 480, (b) with its soma in the molecular layer and its processes extending from the upper part of the molecular layer to the fibre bundles of the granular layer; X 310.



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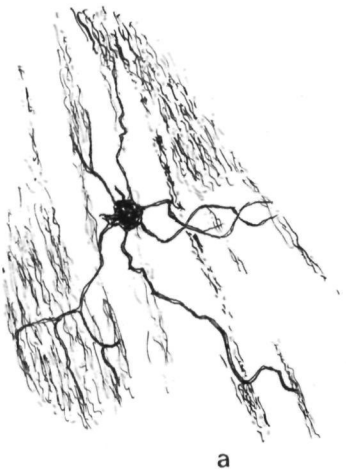
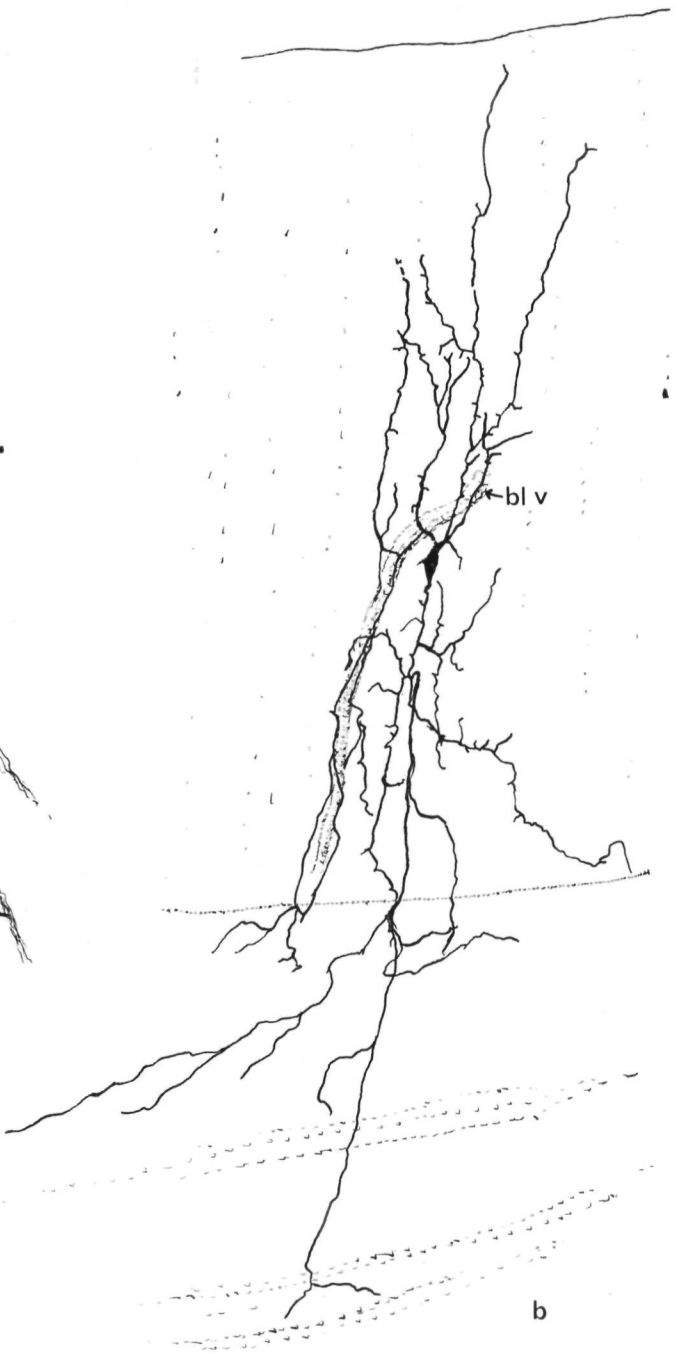


a

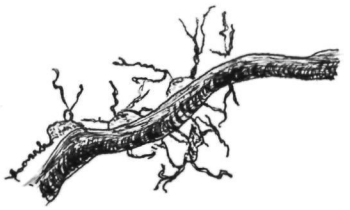


b

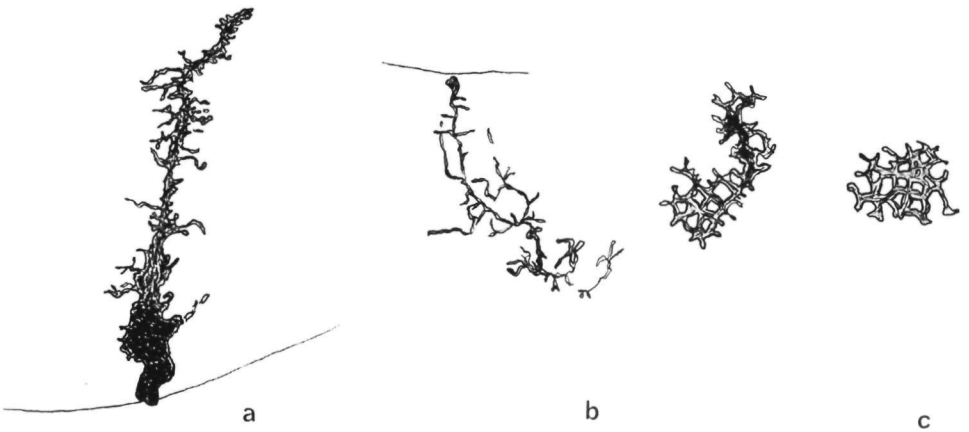
36

Fig. 37. Microglial cells along a blood vessel in the molecular layer of the mature corpus cerebelli. Transverse Golgi preparation. X 480.

Figs. 38 to 40. Stages in the development of neuroglia, as observed in Golgi preparations. X 480. Fig. 38. (a) ependymal cell in the corpus cerebelli; transverse section of a 20 mm trout, (b) processes of an ependymal cell in the corpus cerebelli, reaching the meningeal surface with a terminal knob; transverse section of a 20 mm trout, (c) septa of velate protoplasmic astrocytes in the granular layer of the corpus cerebelli; sagittal section of a 20 mm trout. Fig. 39. (a) velate protoplasmic astrocyte in the granular layer of the corpus cerebelli, showing compartmentalization; sagittal section of a 24 mm trout, (b) velate protoplasmic astrocyte in the granular layer of the corpus cerebelli, showing contact with a blood vessel; transverse section of a 24 mm trout. Fig. 40. Two successive sagittal sections of the valvula cerebelli of a 24 mm trout. Completely drawn neuroglial elements are numbered 1 through 8. The somata of other neuroglial cells are indicated with dotted lines. Further explanation in the text.



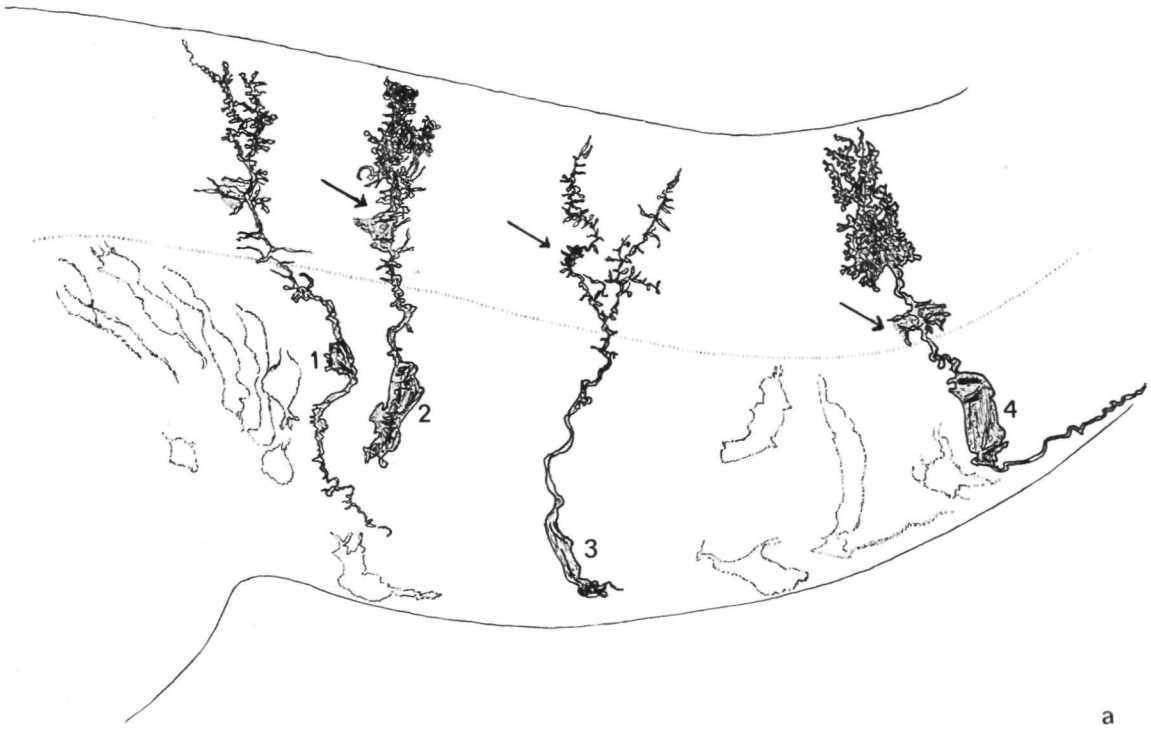
37



38

39


6
5
7
8
b



a

Fig. 41. Matrix cells in the cerebellar anlage of a 5.5 mm embryo. Sagittal section. X 5400.

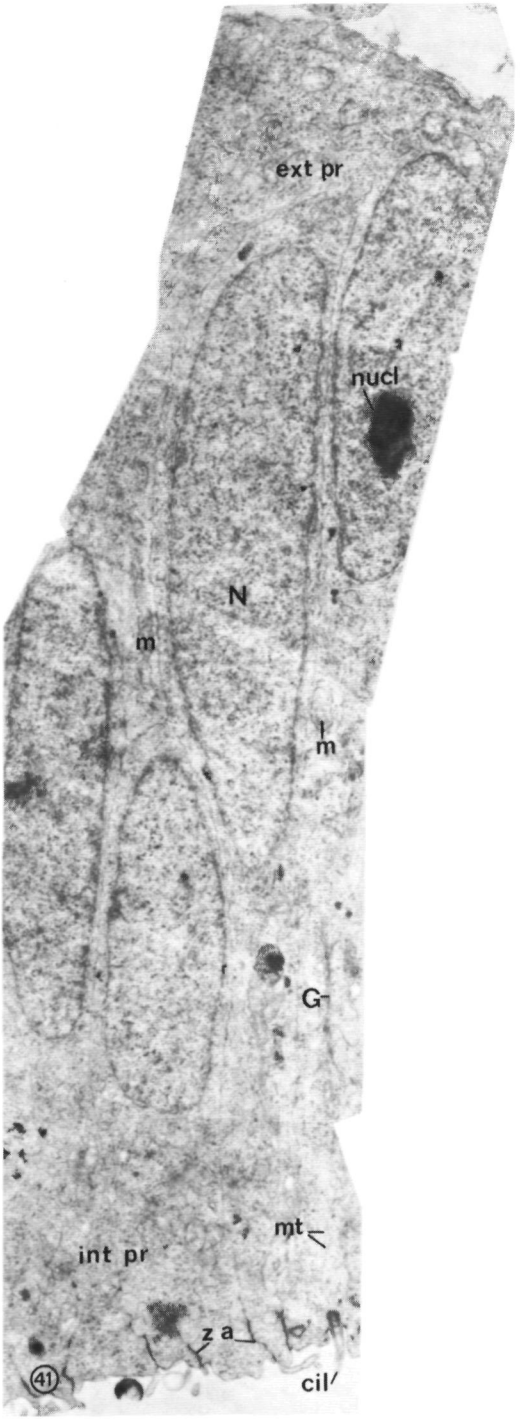
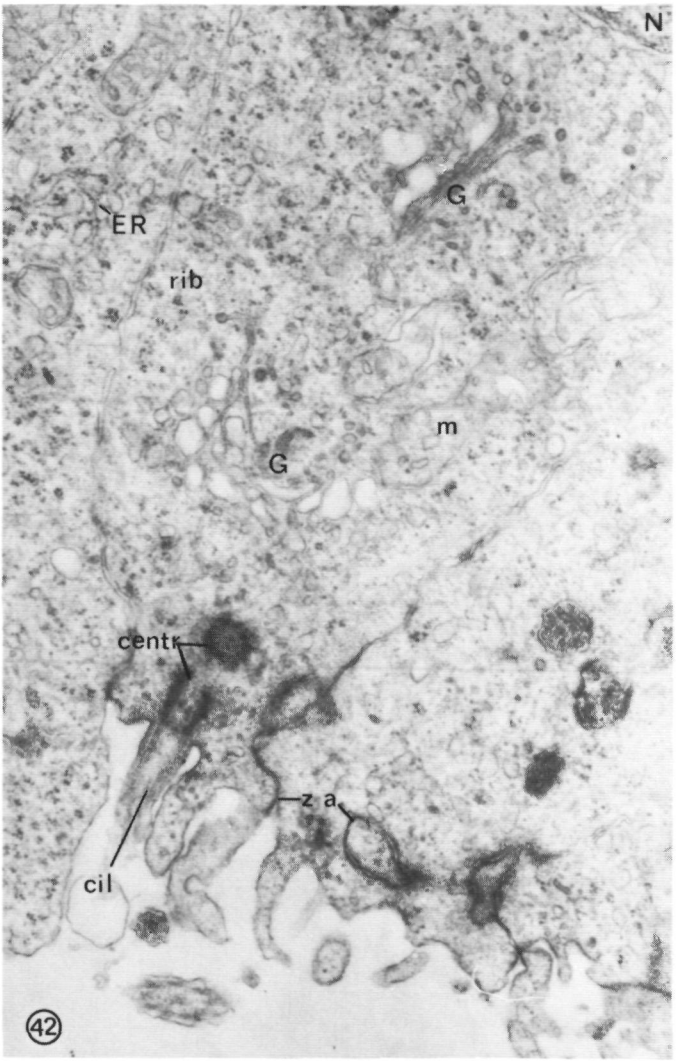

ext pr
nucl
N
m
m
G
mt
int pr
z a
41
cil

Fig. 42. Matrix cells in the cerebellar anlage of a 5.5 mm embryo. Detail of the internal processes. Sagittal section. X 23000.

Fig. 43. The submeningeal region of the cerebellar anlage of a 9.5 mm embryo. Sagittal section. X 12500.


N
G
ER
rib
m
G
centr
z a
cil
42

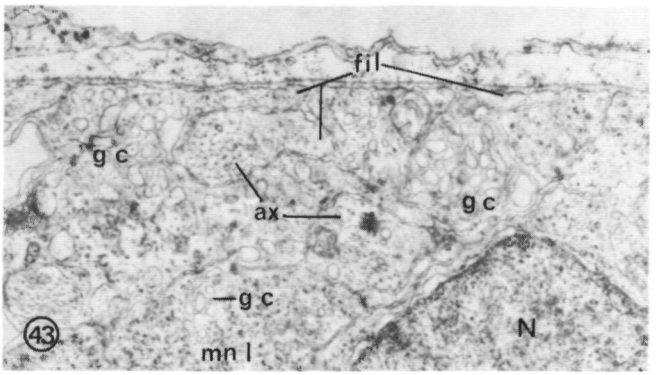
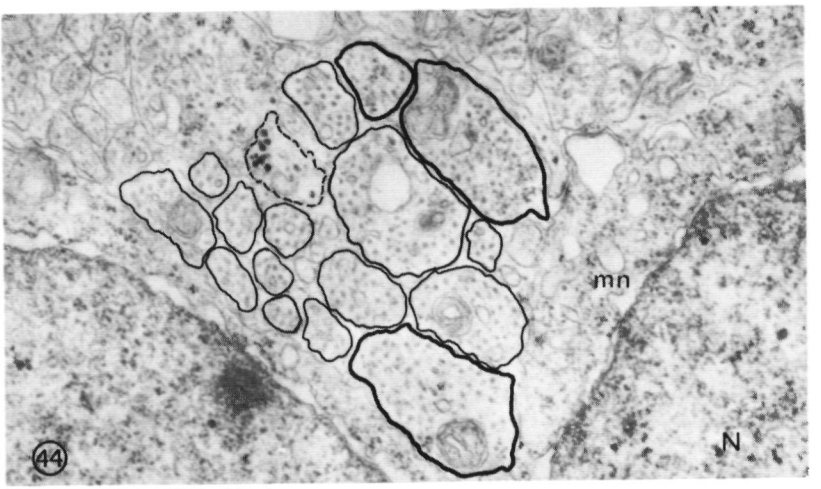

fil
g c
ax
g c
g c
43
N
mn l

Fig. 44. The central area of the lateral thickening of a 11.5 mm embryo. Transverse section. X 25000. Thin lines surround axons, heavy lines dendrites and the interrupted line a glial process.

Fig. 45. A detail of the secondary matrix layer, at some distance from matrixzone M. Transverse section of a 13 mm trout. X 25000. The structures are tangentially oriented. Two types of filaments are shown: a) microfilaments, here in a glial process (gliofilaments, gf), and b) fine filaments (ff), either densely or loosely arranged.


mn
N
44

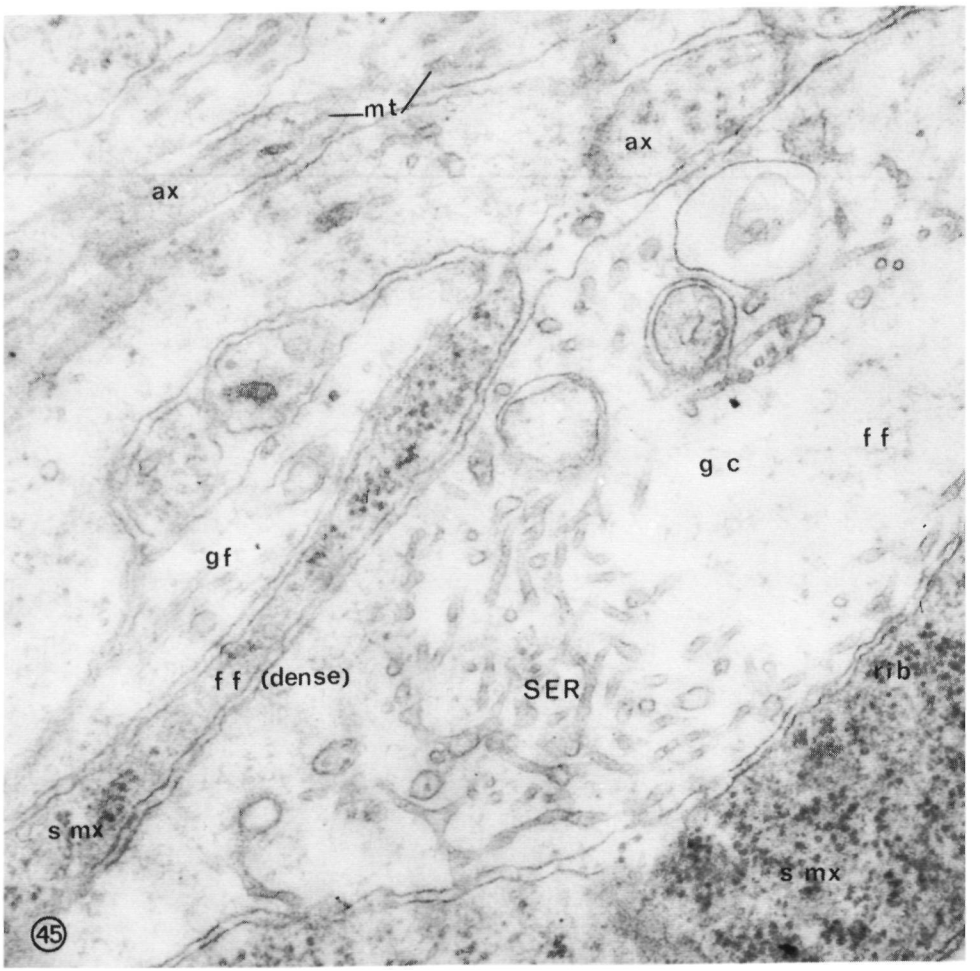

mt
ax
ax
ff
gc
gf
ff (dense)
SER
rib
s mx
s mx
45

Fig. 46. Schematic representation of the development of matrix cells into neuroblasts and glioblasts. Only those structures are depicted, which are of particular importance for the identification of the elements in question. (1) matrix cell of the neuroepithelium, (2) ventricular matrix cell, (3) mantle cell with axon (ax), dendrite (d) and glial process (g p), (4) neuroblast, (5) glioblast. For identification of the somata the following structures serve as criteria: nuclei, RER and free ribosomes, and for identification of the processes: microtubules, microfilaments, SER, free ribosomes and glycogen (auxiliary criterion). Further explanation in the text.

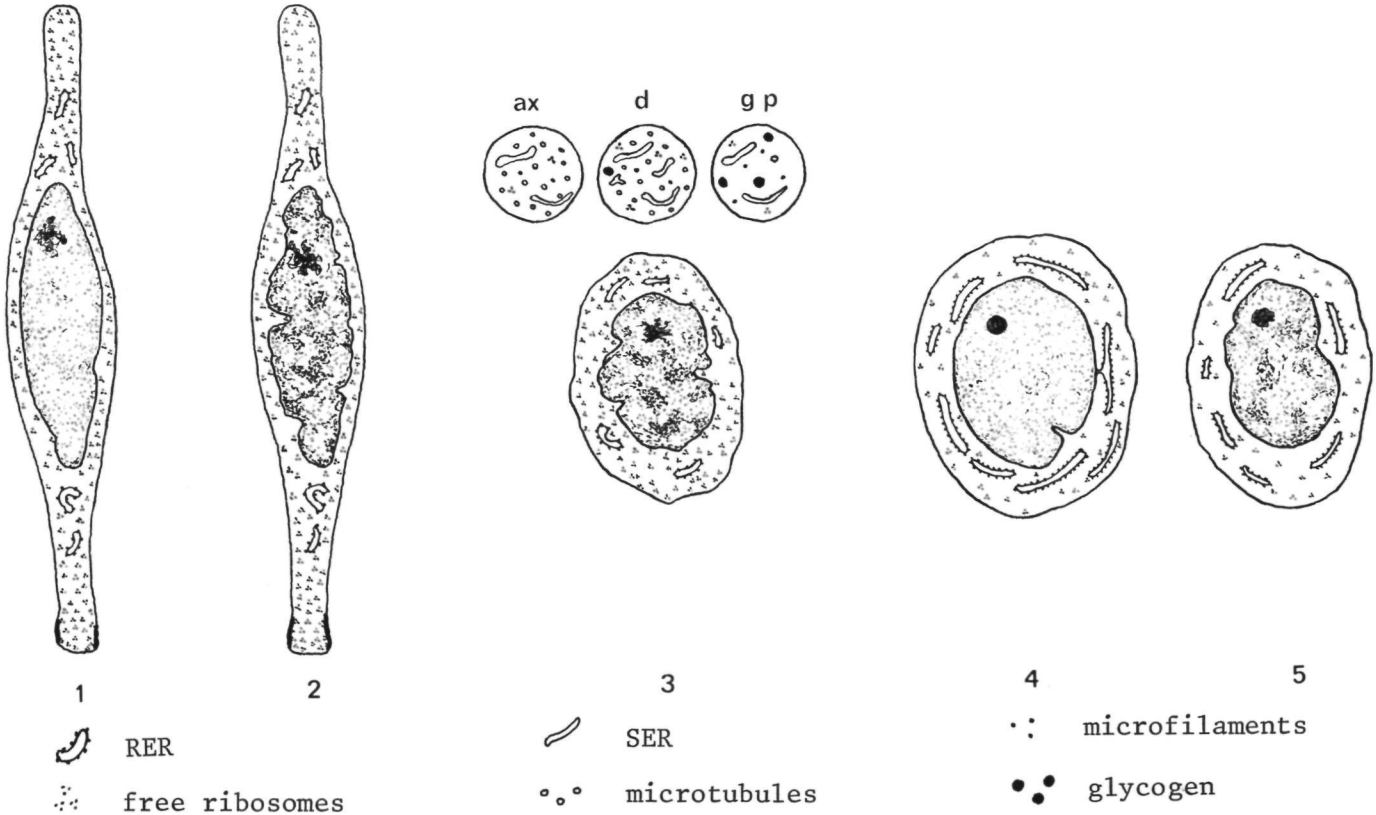

ax
d
g p
1
2
3
4
5
RER
free ribosomes
SER
microtubules
microfilaments
glycogen

Fig. 47. The secondary matrix in the corpus cerebelli, at some distance from matrixzone M. Sagittal section of a 22 mm trout. X 5600. The secondary matrix cell at the left is radially oriented.

Fig. 48. Mitotic secondary matrix cell in the middle of the molecular layer of the corpus cerebelli. Transverse section of a 24 mm trout. X 10800.

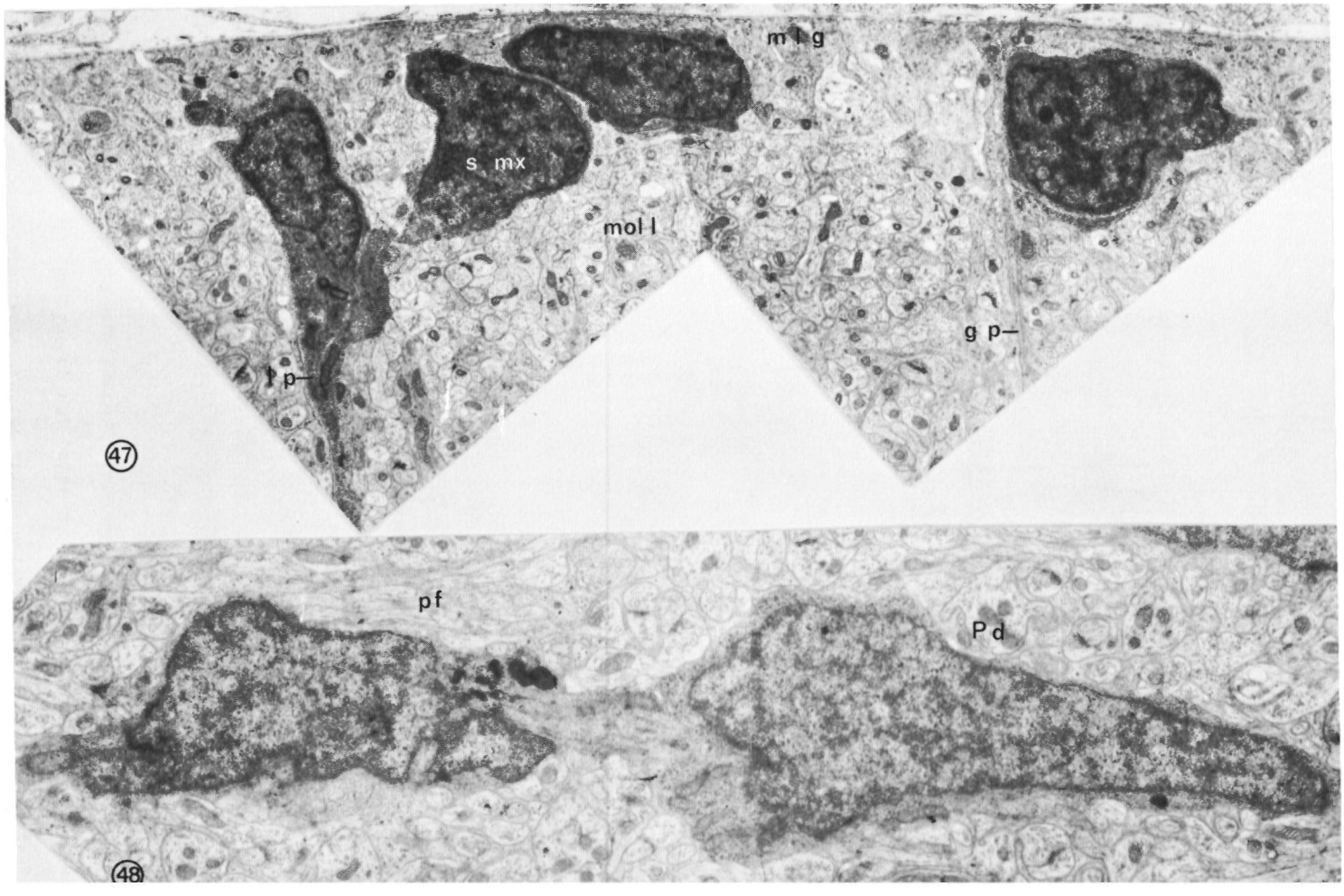
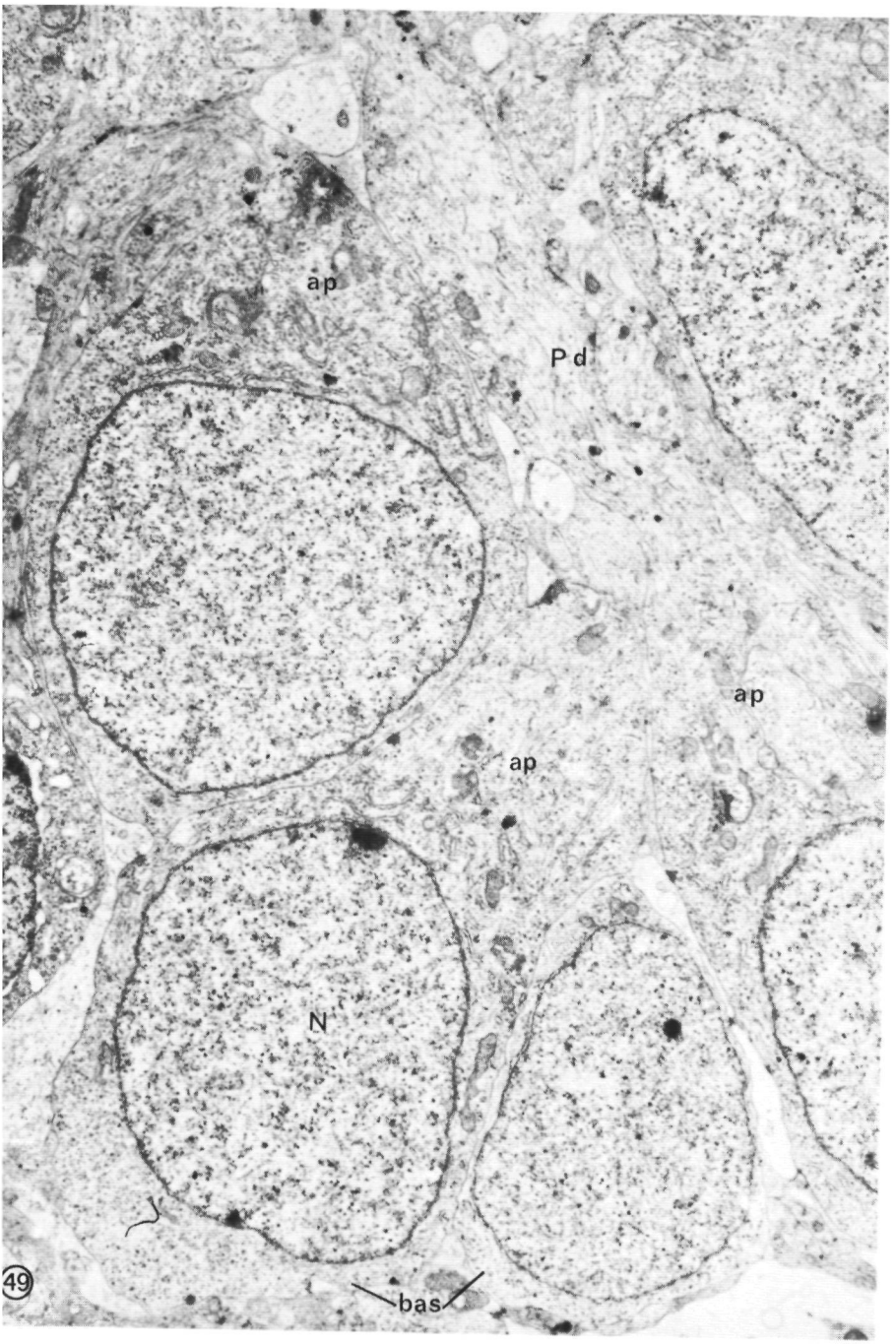

m l g
s mx
mol l
g p—
l p—
47
pf
Pd
48

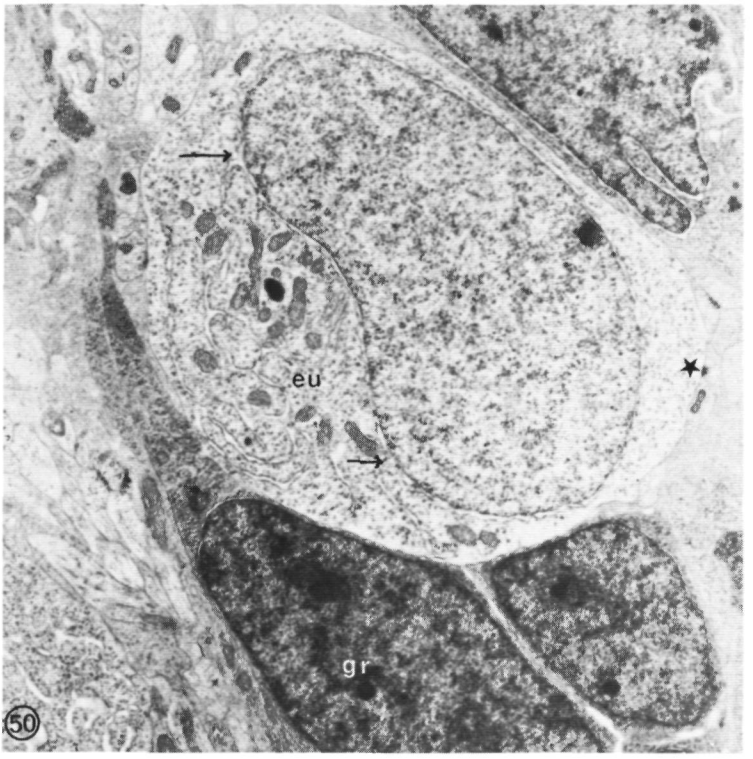
Fig. 49. The ganglionic layer in the corpus cerebelli of a 17 mm trout. Sagittal section. X 5800. The apical parts of the somata are further advanced in development than the basal parts.



49

Fig. 50. A eurydendroid cell and migrating granule cells in the ganglionic layer of the corpus cerebelli. Sagittal section of a 22 mm trout. X 6000. Arrows indicate sites where the RER is continuous with the outer nuclear membrane. A synapse is indicated by an asterisk.

Fig. 51. A climbing fibre synapting with a Purkinje dendrite in the ganglionic layer of the corpus cerebelli. Sagittal section of a 17 mm trout. X 16250.


eu
gr
50

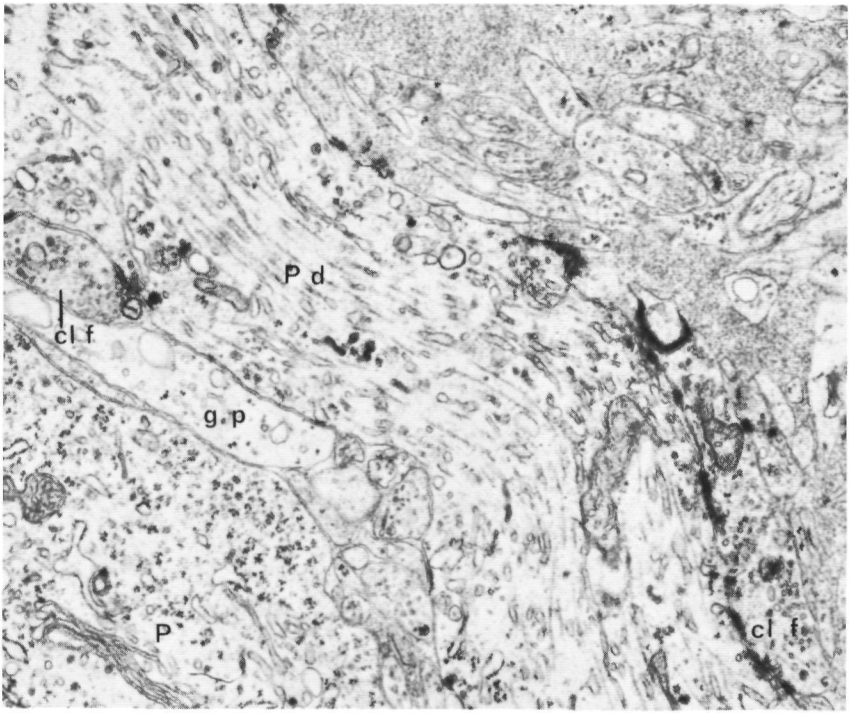

P d
cl f
g p
P
cl f

Fig. 52. Synaptic region in the granular layer of the corpus cerebelli. Sagittal section of a 82 mm trout. X 9300. Some glomeruli are shown. These structures are formed by a mossy fibre rosette, granule cell dendrites and Golgi cell axons, surrounded by the processes of velate protoplasmic astrocytes.

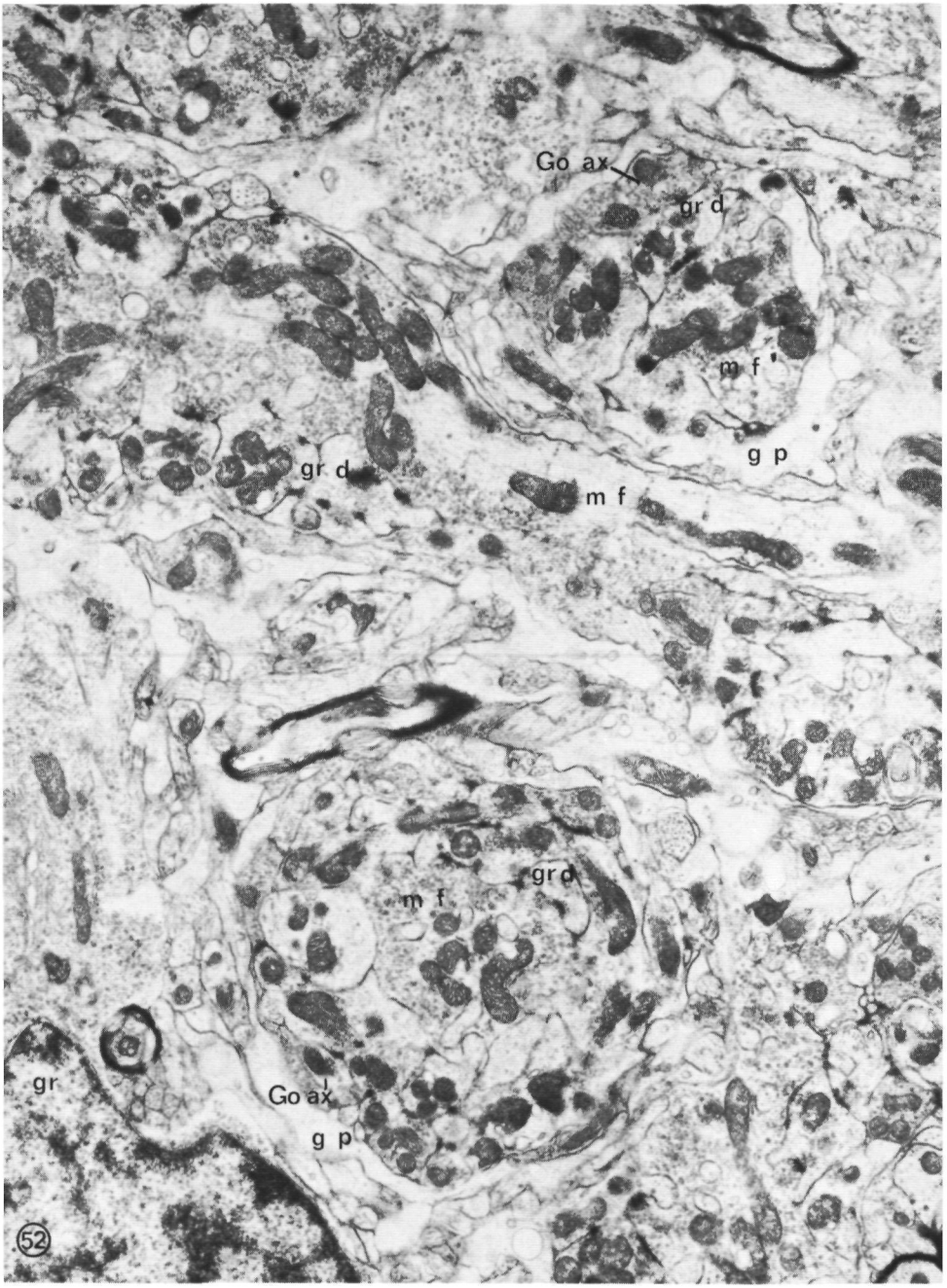

Go ax
gr d
m f
g p
gr d
m f
m f
gr d
gr
Go ax
g p
52

Fig. 53. The median area of the corpus cerebelli in a 82 mm trout. Transverse section. X 2900. Bundles of parallel fibres pierce matrixzone M. The canalis cerebelli is obliterated, hence the layer of astrocytoid ependymal cells borders immediately upon matrixzone M.

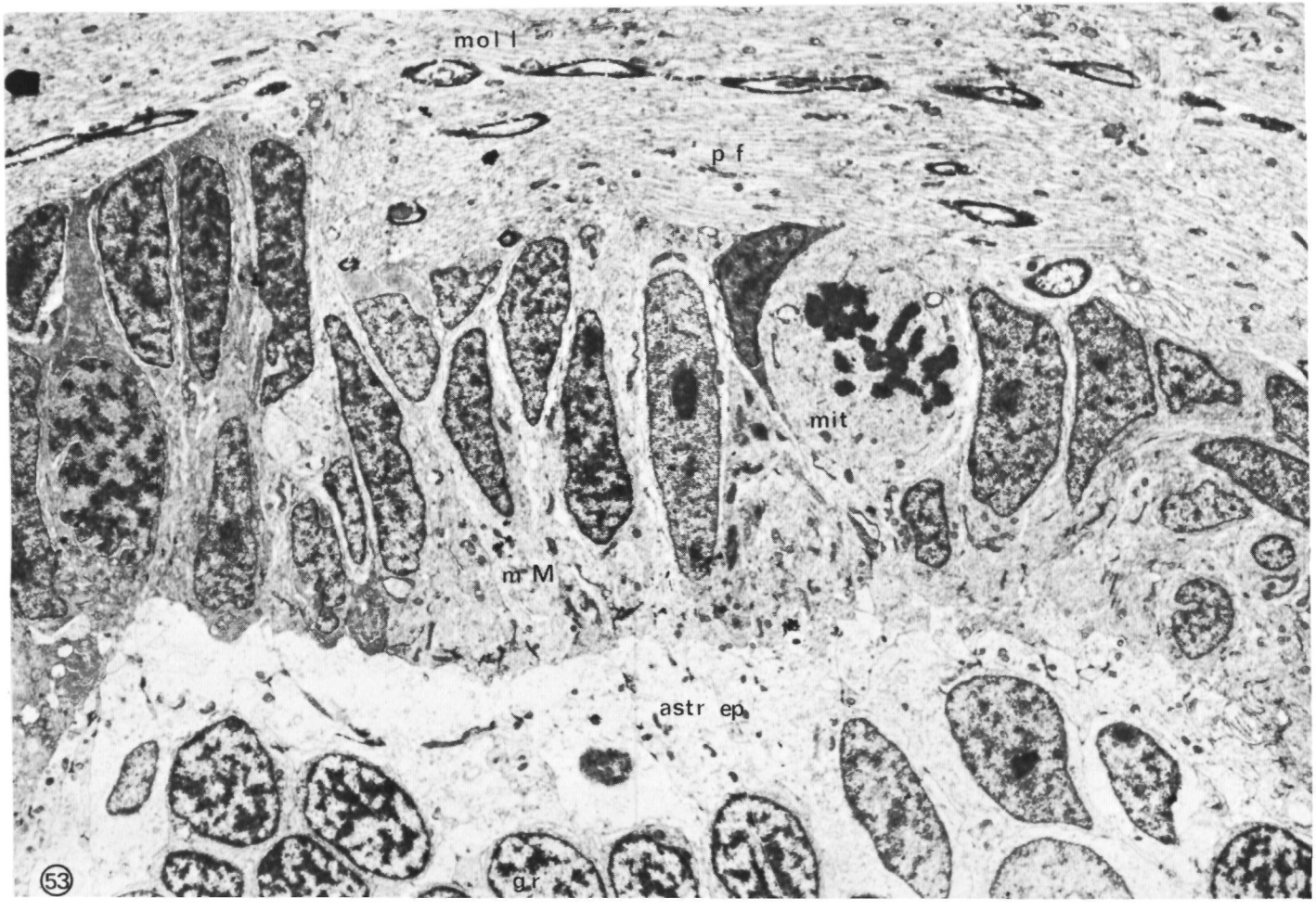
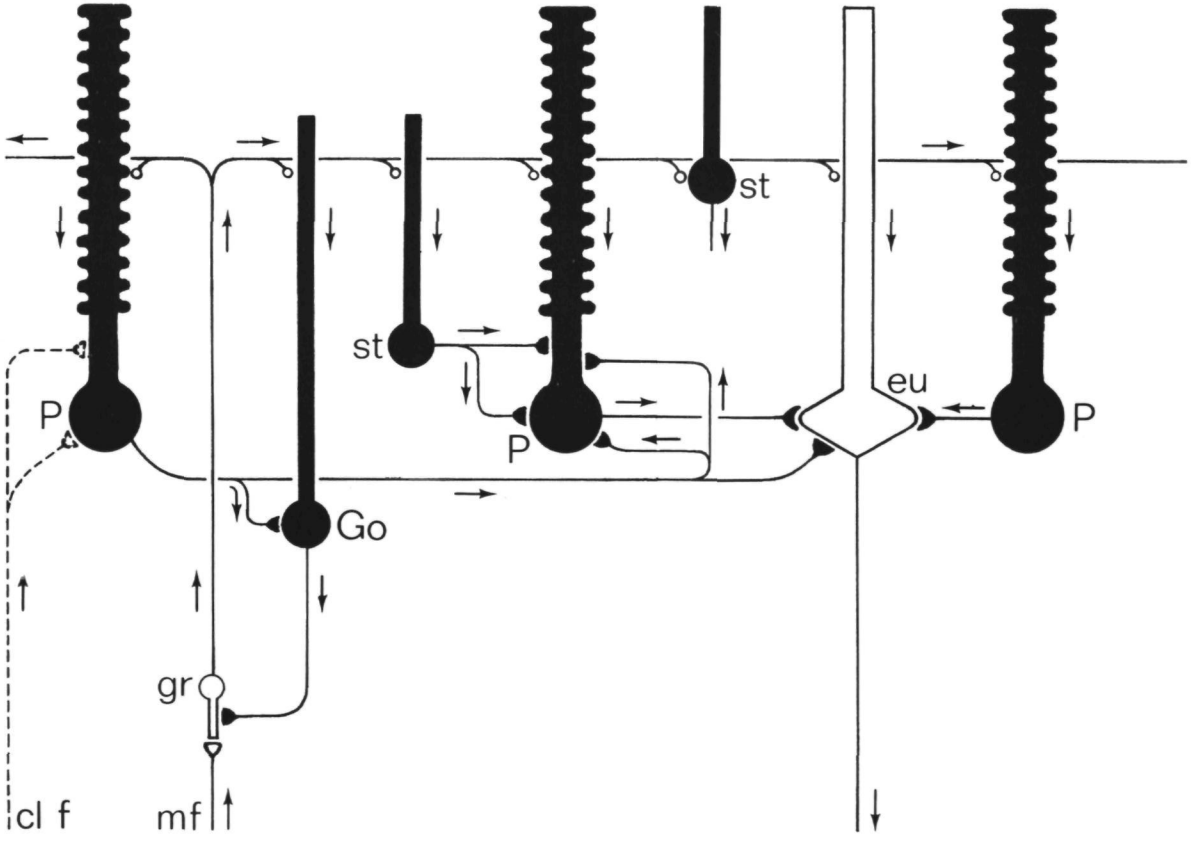

moll
pf
mit
mM
astr ep
gr
53

Fig. 54. Diagrammatic representation of the pattern of connectivity in the corpus cerebelli. The arrows indicate the directions of impulse propagation. Neurons supposed to be inhibitory are shown in black. Structures, only found in young stages so far, are indicated by an interrupted line. Further explanation in the text.


st
st
P
P
P
eu
Go
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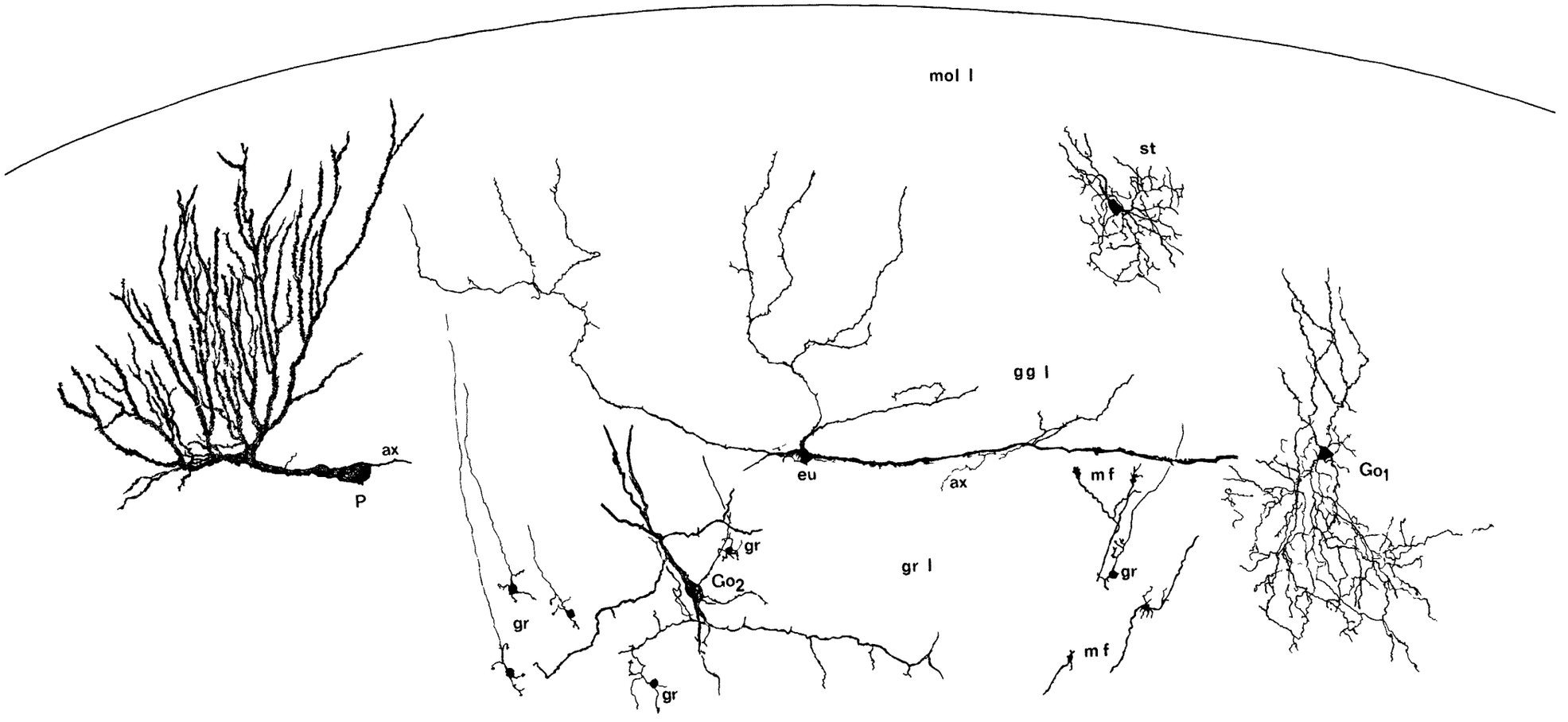
cl f
mf


mol l
st
ax
P
gg l
eu
ax
mf
Go1
gr
Go2
gr l
gr
gr
gr
mf

CURRICULUM VITAE

Erika Pouwels werd op 12 november 1944 te Hilversum geboren. Na het behalen van het diploma gymnasium-B in 1962, begon zij haar studie in de biologie aan de Rijksuniversiteit te Utrecht. In mei 1968 werd het doctoraal examen afgelegd. Van januari 1968 tot december 1969 was zij werkzaam als assistent op het Nederlands Centraal Instituut voor Hersenonderzoek te Amsterdam. In dezelfde periode was zij tevens lerares biologie aan het Christelijk Atheneum in Amsterdam-Oost. Vanaf december 1969 is zij in dienst van het Laboratorium voor Anatomie en Embryologie van de Katholieke Universiteit te Nijmegen.

STELLINGEN

I

De ontwikkeling van de granulaire cellen in het cerebellum, zoals beschreven door Cajal ('11), is niet algemeen geldig.

Cajal, S. Ramon y (1911): Histologie du système nerveux de l'homme et des vertébrés. tome II. Madrid.

II

In het cerebellum van de forel vindt de productie van neuroblasten en glioblasten niet in twee opeenvolgende perioden plaats.

III

De opvatting van Altman ('75) dat tijdens de ontwikkeling van het cerebellum van de rat de gerichte groei van de parallelvezels de ruimtelijke ordening van de glia-uitlopers in de moleculaire laag bepaalt, is onjuist.

Altman, J. (1975): J. Comp. Neur., 163: 427-448

IV

Tussen de opeenvolgende stoornissen, die gedurende de ontwikkeling van het cerebellum bij de zogenoemde weaver mutanten van de muis optreden, behoeft geen direct causaal verband te bestaan.

Rakic, P., R.L. Sidman (1973): J. Comp. Neur., 152: 103-132

V

De mening dat de adenohypophyse van ectodermale, en niet van neurectodermale oorsprong is, is aanvechtbaar.

Takor Takor, T., A.G.E. Pearse (1975): J. Embryol. exp. Morph. 34: 311-325

VI

Astroglia is in staat synaptische regeneratie te stimuleren.

Eccles, J.C. (1976): Naturwissenschaften 63: 8-15

VII

Ten onrechte stellen Schultze e.a. ('74) dat de door hen uitgewerkte methode om met behulp van autoradiografie de duur van de mitotische cyclus te bepalen van diè neuroepitheel cellen, die later tot een bepaald type neuron zullen differentiëren, kwalitatieve gegevens kan opleveren.

Schultze, B., e.a. (1974): J. Comp. Neur. 158: 207-218

VIII

De opvatting dat cytochalasine B zijn werking uitoefent aan het celoppervlak (Bluemink, '71; Sanger en Holtzer, '72), is niet onverenigbaar met de opvatting dat deze stof het systeem van microfilamenten in de cel aantast (Wessells e.a., '71; Yamada e.a., '71).

Bluemink, J.G. (1971): Z. Zellforsch. 121: 102-126

Sanger, J.W., H. Holtzer (1972): Proc. Nat. Acad. Sci. USA 69: 253-257

Wessells, N.K., e.a. (1971): Science 171: 135-143

Yamada, K.M., e.a. (1971): J. Cell Biol. 49: 614-635

IX

Het vóórkomen van grote concentraties kokmeeuwen in West-Europa, zowel in het voortplantingsseizoen als in de winter, is het gevolg van menselijk ingrijpen.

X

De aanwezigheid van gezonde planten in de werkruimte van de onderzoeker heeft een gunstige uitwerking op de voortgang van het wetenschappelijk onderzoek.

8 april 1976 E. Pouwels